# AQUATIC EFFECTS TECHNOLOGY EVALUATION (AETE) PROGRAM

**1997 Field Program**
**Final Report**
**Heath Steele Mine Site,**
**New Brunswick**

**AETE Project 4.1.3**

**September 1998**
**Revised as of March 1999**

101-02626/

# 1997 FIELD PROGRAM - AETE HEATH STEELE

# SITE REPORT

Report prepared for:

Aquatic Effects Technology Evaluation (AETE) Program
Canada Centre for Mineral and Energy Technology (CANMET)
Natural Resources Canada
555 Booth Street
Ottawa, Ontario
K1A 0G1

Prepared by:

BEAK INTERNATIONAL INCORPORATED

14 Abacus Road
Brampton, Ontario
L6T 5B7

and

455 Boul. Fénélon, Suite 104
Dorval, Québec
H9S 5T8

September 1998
Revised as of March 1999

# 1997 FIELD PROGRAM - AETE
# HEATH STEELE
# SITE REPORT


**BEAK INTERNATIONAL INCORPORATED**

**PROJECT TEAM:**

Biostatistics: Donald R. Hart, Alan J. Burt
Field Crew Leader: Robert Eakins
Data Management: Deb McMillan
Toxicology: Julie Schroeder

**PROJECT MANAGEMENT TEAM**

Paul McKee, M.Sc.

Dennis Farara, B.Sc.

## AQUATIC EFFECTS TECHNOLOGY EVALUATION PROGRAM

## Notice to Readers

---

### 1997 AETE Field Program
### Heath Steele Mine Site, New Brunswick

The Aquatic Effects Technology Evaluation (AETE) program was established to review appropriate technologies for assessing the impacts of mine effluents on the aquatic environment. AETE is a cooperative program between the Canadian mining industry, several federal government departments and a number of provincial governments; it is coordinated by the Canada Centre for Mineral and Energy Technology (CANMET). The program was designed to be of direct benefit to the industry, and to government. Through technical and field evaluations, it identified cost-effective technologies to meet environmental monitoring requirements. The program included three main areas: acute and sublethal toxicity testing, biological monitoring in receiving waters, and water and sediment monitoring.

The technical evaluations are conducted to document certain tools selected by AETE members, and to provide the rationale for doing a field evaluation of the tools or provide specific guidance on field application of a method. In some cases, the technical evaluations included a go/no go recommendation that AETE takes into consideration before a field evaluation of a given method is conducted.

The technical evaluations are published although they do not necessarily reflect the views of the participants in the AETE Program. The technical evaluations should be considered as working documents rather than comprehensive literature reviews. The purpose of the technical evaluations was to document specific monitoring tools. AETE committee members would like to stress that no one single tool can provide all the information required for a full understanding of environmental effects in the aquatic environment.

For more information on the monitoring techniques, the results from their field application and the final recommendations from the program, please consult the AETE Synthesis Report to be published in the spring of 1999.

Any comments concerning the content of this report should be directed to:

Geneviève Béchard
Manager, Metals and the Environment Program
Mining and Mineral Sciences Laboratories - CANMET
Room 330, 555 Booth Street, Ottawa, Ontario, K1A 0G1
Tel.: (613) 992-2489 Fax: (613) 992-5172
E-mail: gbechard@nrcan.gc.ca

# EXECUTIVE SUMMARY

The Heath Steele (New Brunswick) mine study is one of four field evaluations carried out in 1997 under the Aquatic Effects Technology Evaluation (AETE) Program, a joint government-industry program to evaluate the cost-effectiveness of technologies for the assessment of mining-related impacts in the aquatic environment. The other three mines studied were Dome (Ontario), Mattabi (Ontario) and Myra Falls (British Columbia). Results of all four studies are summarized and evaluated in a separate summary report.

Heath Steele Division, Noranda Inc., is a base metal mine located northwest of Miramichi, in the Tomogonops River headwaters, which flows into the Northwest Miramichi River. The mine produces zinc, lead, copper and silver concentrations. The mine was first developed in the mid-1950s and, although great strides have been made in cleanup of acid rock drainage (ARD) and metal leaching problems in recent years, the mine continues to release substantial loadings of metals to the Little South Branch Tomogonops River due to the effects of ARD. These loadings are being progressively reduced.

The 1997 field studies were carried out in the Little South Branch Tomogonops River and downstream in the main Tomogonops River, upstream of any effect of the treated effluent from the tailings pond. Sampling was carried out here rather than downstream of the effluent because this reach offered an opportunity for study in a stronger water quality gradient. Sampling was not extended downstream of the treated effluent because of the confounding effects of a greatly increased water hardness in the river produced by calcium added in the form of lime for effluent treatment. The entire section of river studied here consists of riffle/run habitat, with a rock-cobble substrate.

The objectives of the 1997 field program were to test 13 hypotheses formulated under four guiding questions:

1. are contaminants getting into the system (and to what degree and in which compartments)?
2. are contaminants bioavailable?
3. is there a measurable (biological) response? and
4. are contaminants causing the responses?

The hypotheses are more specific questions about the ability or relative ability of different monitoring tools to answer these four general questions about mine effect. The evaluation of tools included: sediment monitoring (sediment toxicity tests); fish monitoring (tissue metallothionein and metal analyses, and population/community indicators), and; integration of tools (relationships between exposure and biological responses and use of effluent sublethal toxicity).

Of the 13 hypotheses, 8 were tested at Heath Steele as outlined in Table 1.1. The hypotheses not tested at Heath Steele include tissue comparisons of metals and metallothionein contents (because resident fish are small and analyses were of whole gut rather than individual organs), and sediment-related hypotheses because no soft sediment occurs in the affected reach. One sediment-related hypothesis was tested at Heath Steele (linkage between benthos and sediment quality) using periphyton as a surrogate for sediment.

**Study Design**

The study design at Heath Steele was based on river sampling for fish and benthos using a gradient design, including five exposure reaches and three reference reaches, with each reach consisting of two stations. Each exposure reach along the gradient had a different concentration of metals, with the key metals being zinc, copper, cadmium and lead. Three reference reaches were established to span the range of river size represented across the exposure gradient.

**Sampling Program**

The field survey at Heath Steele was completed in August 1997, and included:

- water sampling at each of 16 stations (8 reaches) where fish and benthos were sampled;
- benthic sampling at each of 16 stations (2 samples at each) using a T-sampler;
- periphyton sampling at each of 16 stations (2 samples at each) carried out by scraping of rock substrate surfaces;
- fish population and community at each of 16 stations using a standard electrofishing effort;
- collection of up to 134 juvenile Atlantic salmon and 47 blacknose dace for measurement of length, weight and age (by length frequency analysis with confirmatory aging). Some stations produced no salmon or dace, due to apparent toxicity at the most exposed sites and in one instance due to habitat limitations (fish migration barriers);
- collection of four viscera samples (where possible) per station from wild juvenile salmon (one fish per sample), one to six composite blacknose dace per station and variable numbers of brook trout at each station. In addition, two samples of viscera were collected from each of two caged juvenile salmon from a nearby salmon rearing facility exposed at each station for nine days; and
- three "effluent" samples for chronic toxicity testing using the *Ceriodaphnia dubia* survival and reproduction test, the fathead minnow survival and growth test, the *Selenastrum capricornutum* growth test and the *Lemna minor* growth test. "Effluent" consisted of water collected from the Little South Branch Tomogonops River at the location most affected by the mine and routinely monitored by mine personnel.

**Data Overview**

***Water Quality***

Total and dissolved (0.45 µm-filtered) concentrations of Zn, Cd, Pb, Cu, Al and Fe all showed concentration gradients downstream of Heath Steele. All of these parameters except Al remained elevated relative to reference site concentrations at the downstream extent of the exposure gradient, and all occurred in excess of Canadian Water Quality Guidelines in some or all exposure reaches (depending on the metal). Dissolved and total metal concentrations were similar for Zn, Cu and Cd, while dissolved Al, Fe and Pb were substantially lower than their total concentrations.

***Periphyton***

Periphyton samples were rich in species and variable in biomass, and no trends were observed in response to the water quality gradient or between exposed and reference reaches. In terms of metal concentrations in periphyton, exposed periphyton contained greater levels of Cd, Cu, Zn and Pb, although only Pb in periphyton appeared to track the water quality gradient in the exposure reaches.

***Benthic Macroinvertebrates***

Benthic community structure responded to the water quality gradient, with exposed stations showing reduced total numbers of taxa and reduced numbers of Ephermoptera, Plecoptera and Trichoptera (EPT) taxa. Trends were also observed in apparent indicator taxa such as *Micropsectra* which was sensitive to high degrees of exposure, and *Rheocricotopus* which was most abundant at higher degrees of exposure. Total benthic density, however, appeared unresponsive to metal exposure.

***Fish***

Fish community structure varied from reach to reach, with juvenile Atlantic salmon densities suppressed upstream of the most downstream exposure reach, apparently due to a partial migration barrier. Ten species were represented in the collections, with juvenile salmon, blacknose dace, lake chub and brook being the most common. No fish were found at the most exposed station, apparently due to toxicity.

Catch-per-unit-effort (numbers) and biomass-per-unit-effort (all species) clearly responded to the exposure gradient, and gradually increased from upstream to downstream.

Fish size at age appeared unresponsive to exposure, although Atlantic salmon fry were larger in the exposure area than in the reference area. This effect is probably attributed to higher fry densities and greater competition in the reference area.

Metallothionein (MT) levels in fish viscera were greater in exposed salmon and dace than in reference fish. MT concentrations in caged juvenile salmon viscera and gill closely tracked metal concentrations in water after the exposure period.

Visceral metal levels appeared elevated in exposed wild fish for some metals, although this response was less evident in caged fish.

*Effluent Toxicity*

All effluent samples tested were chronically toxic to *Ceriodaphnia, Selenastrum* and *Lemna*, while sublethal and lethal toxicity occurred in two of the three tests in fathead minnow. The degree of toxicity corresponded with metal concentration in *Ceriodaphnia* and *Selenastrum*, while *Lemna* and fathead minnow responses did not appear to track metal concentrations in the samples.

**Hypothesis Testing**

Hypothesis testing results are summarized in Table 5.2. Results of testing indicate that some of the metals are bioavailable, that biological responses occur in both benthos and fish, and that metals appear to cause some of these responses.

**Technology Evaluation**

Many of the monitoring tools evaluated at Heath Steele demonstrated a mine effect. Periphyton community structure, fish growth and benthic community density were ineffective. Those tools that demonstrated mine effects or partially demonstrating mine effects included water quality, periphyton metals, fish viscera and gill metals and MT, fish population/community indicators, effluent chronic toxicity and benthic community indicators. Table 6.2 summarizes the effectiveness of the various tools tested at Heath Steele.

Among those tools compared in hypothesis testing, some appeared more effective than others. Table 6.3 provides a summary of tool comparisons.

Conclusions on the cost-effectiveness of the tools based on results from all four mine sites studied in 1997 are found in a separate document "Summary and Cost-Effectiveness Evaluation of Aquatic Effects Monitoring Technologies Applied in the 1997 AETE Field Evaluation Program".

# SOMMAIRE

L'étude du site de la mine Heath Steele (Nouveau-Brunswick) est l'une des quatre évaluations sur le terrain effectuées en 1997 dans le cadre du Programme d'évaluation des techniques de mesure d'impacts en milieu aquatique (ETIMA), programme conjoint gouvernement-industrie destiné à évaluer le rapport coût-efficacité des technologies d'évaluation des impacts liés aux activités minières dans le milieu aquatique. Les trois autres sites miniers étudiés étaient ceux de Dome (Ontario), de Mattabi (Ontario) et de Myra Falls (Colombie-Britannique). On présente un résumé et une évaluation des résultats de ces quatre études dans un rapport sommaire distinct.

La division Heath Steele de Noranda Inc. est une mine de métaux communs située au nord-ouest de Miramichi, dans le bassin du cours supérieur de la rivière Tomogonops, qui se jette dans la partie nord-ouest de la rivière Miramichi. Depuis le milieu des années 50, cette mine produit du zinc, du plomb, du cuivre et de l'argent et, malgré les efforts considérables faits au cours des dernières années pour éliminer les eaux d'exhaure acides (EEA) et pour régler des problèmes de lixiviation de métaux, cette exploitation minière continue de rejeter d'importantes charges de métaux dans le bras Little South de la rivière Tomogonops à cause des EEA. On travaille à réduire progressivement ces charges.

En 1997, on a effectué les études sur le terrain dans le bras Little South de la rivière Tomogonops et en aval du cours principal de la rivière Tomogonops, en amont du point de rejet de l'effluent traité du bassin de décantation des résidus. On a effectué l'échantillonnage à cet endroit plutôt qu'en aval de l'effluent parce que ce tronçon rendait possible une étude dans un gradient de qualité de l'eau plus étendu. On n'a pas étendu l'échantillonnage à la zone en aval du point de rejet de l'effluent traité à cause d'effets venant brouiller les indices; en effet, l'addition de calcium sous forme de chaux pour le traitement de l'effluent entraîne une forte augmentation de la dureté de l'eau dans la rivière. Toute cette section de la rivière étudiée consiste en un habitat de zones de courant et de rapides à substrat de roches et de galets.

Les objectifs du programme sur le terrain de 1997 étaient de vérifier 13 hypothèses formulées pour tenter de répondre à quatre questions principales :

1. Est-ce que les contaminants pénètrent dans le réseau aquatique (et dans l'affirmative, dans quelle mesure et dans quels compartiments)?
2. Les contaminants sont-ils biodisponibles?
3. La réponse (biologique) est-elle mesurable?
4. Les contaminants sont-ils la cause de ces réponses?

Ces hypothèses représentent des questions plus spécifiques concernant la capacité (relative) des différents outils de surveillance de répondre à ces quatre questions générales sur les effets des activités minières. L'évaluation des outils prévoyait notamment la surveillance des sédiments (tests de toxicité des sédiments), la surveillance des poissons (dosage de la métallothionéine et des métaux des tissus et détermination des indicateurs des populations/communautés) et, enfin, l'intégration des outils (rapports entre l'exposition et les réponses biologiques et utilisation de la toxicité sublétale des effluents).

On a vérifié 8 des 13 hypothèses au site de la mine Heath Steele (voir le tableau 1.1.). Les hypothèses non vérifiées à ce site sont notamment les comparaisons des teneurs en métaux et en métallothionéine des tissus (parce que les poissons qui y résident sont petits et qu'on utilisait l'ensemble des entrailles plutôt que des organes particuliers pour les analyses), ainsi que les hypothèses concernant les sédiments parce qu'on ne trouve pas de sédiments meubles dans le bief touché. On a testé une hypothèse concernant les sédiments au site Heath Steele (rapport entre le benthos et la qualité des sédiments) en utilisant le périphyton comme substitut pour les sédiments.

**Plan de l'étude**

Le plan de l'étude au site Heath Steele était basé sur l'échantillonnage des poissons et du benthos de la rivière selon un gradient, et il comportait cinq tronçons d'exposition et trois tronçons de référence, chacun comportant deux stations. À l'intérieur du gradient, chacun des tronçons était caractérisé par différentes concentrations de métaux, dont les principaux sont le zinc, le cuivre, le cadmium et le plomb. On a choisi trois tronçons de référence de façon à représenter la gamme des largeurs de la rivière correspondant au gradient d'exposition.

**Programme d'échantillonnage**

On a terminé les relevés sur le terrain pour le site Heath Steele en août 1997, notamment :

- l'échantillonnage de l'eau à chacune des 16 stations (8 tronçons) où l'on a échantillonné les poissons et le benthos;
- l'échantillonnage du benthos à chacune des 16 stations (2 échantillons par station) à l'aide d'un échantillonneur en T;
- l'échantillonnage du périphyton à chacune des 16 stations (2 échantillons par station), prélevés en grattant la surface de substrats rocheux);
- la détermination des populations et des communautés de poissons à chacune des 16 stations à l'aide d'une méthode normalisée de pêche électrique;
- la collecte de jusqu'à 134 juvéniles de saumon de l'Atlantique et de 47 naseux noirs pour les mesures de longueur, de poids et d'âge (par analyse des fréquences de longueur avec confirmation par l'âge). Dans certaines stations, on n'a prélevé ni saumons ni naseux à cause de la toxicité apparente observée dans la plupart des sites exposés et, dans un cas, à cause des limites de l'habitat (barrières entravant la migration des poissons);
- la collecte de 4 échantillons de viscères de juvéniles de saumon par station (si possible) (un poisson par échantillon), de 1 à 6 échantillons composés de naseux noirs par station et de nombres variables d'ombles de fontaine à chaque station. De plus, on a obtenu deux échantillons de viscères de chacun des deux juvéniles de saumon en cage provenant d'une écloserie voisine, après une exposition de 9 jours à deux stations;

- la collecte de trois échantillons d'« effluent » pour des tests de toxicité chronique basés sur le test de survie et de reproduction de *Ceriodaphnia dubia*, le test de survie et de croissance de la tête-de-boule, le test de croissance de *Selenastrum capricornutum* et le test de croissance de *Lemna minor*. L'« effluent » était constitué d'eau recueillie dans la bras Little South de la rivière Tomogonops, à l'endroit le plus touché par les activités minières, et surveillé de façon régulière par le personnel de la mine.

**Aperçu des données**

***Qualité de l'eau***

Les concentrations de Zn, Cd, Pb, Cu, Al et Fe totaux et dissous (après traitement avec un filtre à mailles de 0,45 µm) indiquaient toutes la présence de gradients de concentration en aval du site Heath Steele. Tous ces paramètres sauf Al restait élevés par rapport aux concentrations des sites de référence à l'extrémité aval du gradient d'exposition, et tous dépassaient les limites des Recommandations pour la qualité des eaux du Canada dans certains des tronçons d'exposition ou dans la totalité de ceux-ci (selon le métal). Les concentrations de métaux dissous et totaux étaient semblables dans le cas du Zn, du Cu et du Cd, alors que celles de l'Al, du Fe et du Pb dissous étaient beaucoup plus faibles que leurs concentrations totales.

***Périphyton***

Les échantillons de périphyton étaient riches en espèces et leur biomasse était variable; on n'a observé aucune tendance en réponse au gradient de qualité de l'eau ou entre les tronçons exposés et les tronçons de référence. Pour ce qui est des concentrations de métaux, le périphyton exposé contenait de plus fortes teneurs en Cd, Cu, Zn et Pb, bien que seule la teneur en Pb du périphyton semblait correspondre au gradient de qualité de l'eau dans les biefs exposés.

***Macroinvertébrés benthiques***

La structure de la communauté benthique variait selon le gradient de qualité de l'eau; en effet, on observait, dans les stations exposées, des nombres totaux réduits de taxons et des nombres réduits des taxons ***Ephemeroptera***, ***Plecoptera*** et ***Trichoptera*** (EPT). On a également observé des tendances chez des taxons considérés comme des indicateurs apparents, par exemple *Micropsectra*, qui est sensible aux fortes expositions, et *Rheocricotopus*, qui était abondant aux fortes expositions. Toutefois, la densité benthique totale ne semblait pas répondre à l'exposition aux métaux.

***Poissons***

La structure des communautés de poissons variait d'un tronçon à l'autre, les densités des juvéniles de saumon de l'Atlantique étant absents de la partie amont de la plupart des tronçons d'exposition en aval, ce qui s'expliquait apparemment par la présence d'une barrière partielle entravant la migration. Dix espèces étaient représentées dans les collections, les plus communes étant les juvéniles de saumon, les naseux noirs, les ménés de lac et les ombles de fontaine. On n'a observé aucun poisson à la station la plus exposée, sans doute à cause de la toxicité.

Les prises par unité d'effort (nombres) et la biomasse par unité d'effort (toutes espèces confondues) correspondaient nettement au gradient d'exposition et augmentaient graduellement d'amont en aval.

La taille des poissons selon l'âge ne semblait pas touchée par l'exposition, même si les alevins de saumon de l'Atlantiques étaient plus gros dans la zone d'exposition que dans la zone de référence. Cet effet est probablement dû aux densités d'alevins plus élevées et à une plus forte compétition dans la zone de référence.

Les teneurs en métallothionéine (MT) dans les viscères des poisson étaient plus élevées chez les saumons et les naseux exposés que chez les poissons de référence. Les concentrations de MT dans les viscères et les branchies des juvéniles de saumon en cage correspondaient assez bien aux concentrations de métaux dans l'eau après la période d'exposition.

Les concentrations de certains métaux dans les viscères semblaient élevées chez les poissons sauvages exposés pour certains métaux, même si cette réponse était moins évidente chez les poisson en cage.

***Toxicité des effluents***

Dans tous les échantillons d'effluents testés, on observait une toxicité chronique pour *Ceriodaphnia, Selenastrum* et *Lemna*, ainsi qu'une toxicité sublétale et létale pour deux des trois tests utilisés avec la tête-de-boule. Le degré de toxicité correspondait à la concentration de métaux chez *Ceriodaphnia* et *Selenastrum*, alors que les réponses de *Lemna* et des têtes-de-boules ne semblaient pas correspondre aux concentrations des métaux dans les échantillons.

**Vérification des hypothèses**

Les résultats des vérifications des hypothèses sont résumés au tableau 5.2; ils indiquent que certains des métaux sont biodisponibles, qu'on observe des réponses biologiques dans le benthos et chez les poissons, et que les métaux semblent être la cause de certaines de ces réponses.

**Évaluation des techniques**

Beaucoup d'outils de surveillance évalués au site Heath Steele indiquaient l'existence d'effets dus aux activités minières. Les outils basés sur la structure des communautés de périphyton, la croissance des poissons et la densité des communautés benthiques n'étaient pas efficaces. Les outils sensibles aux effets des activités minières, même de façon partielle, étaient notamment ceux qu'on utilise pour déterminer la qualité de l'eau, les métaux du périphyton, les teneurs en métaux et en MT des viscères et des branchies des poissons, les indicateurs des populations ou des communautés de poissons, ainsi que les indicateurs de la toxicité chronique des effluents et ceux de la communauté benthique. Le tableau 6.2 résume les données sur l'efficacité des divers outils testés sur le site Heath Steele.

Certains des différents outils comparés pour la vérification des hypothèses semblent plus efficaces que d'autres. Le tableau 6.3 présente un résumé des comparaisons entre ces outils.

Un document distinct, « Summary and Cost-Effectiveness Evaluation of Aquatic Effects Monitoring Technologies Applied in the 1997 AETE Evaluation Program », présente les conclusions sur le rapport coût-efficacité de ces outils, qui sont basées sur les résultats obtenus pour les quatre sites miniers étudiés en 1997.

# 1.0 INTRODUCTION

The Assessment of the Aquatic Effects of Mining in Canada (AQUAMIN), initiated in 1993, evaluated the effectiveness of Canada's *Metal Mining Liquid Effluent Regulations* (MMLER). One of the key recommendations of the 1996 AQUAMIN Final Report is that a revised MMLER include a requirement that metal mines conduct Environmental Effects Monitoring (EEM), to evaluate the effects of mining activity on the aquatic environment, including fish, fish habitat and the use of fisheries resources.

In parallel, the Canada Centre for Mineral and Energy Technology (CANMET) is coordinating a cooperative government-industry program, the Aquatic Effects Technology Evaluation (AETE) program, to review and evaluate technologies for the assessment of mining-related impacts on the aquatic environment. The intention of the AETE program is to evaluate and identify cost-effective technologies to meet environmental monitoring requirements at mines in Canada. The program is focused on evaluation of environmental monitoring tools that may be used for a national mining EEM program, baseline assessments or general impact studies.

The three principal components of the AETE program are lethal and sublethal toxicity testing of water/effluents and sediments, biological monitoring in receiving waters, and water and sediment chemistry assessments. The program includes both literature-based technical evaluations and comparative field programs at candidate sites. The AETE program is presently at the stage of evaluating selected monitoring methods at four case study sites across Canada.

An AETE Pilot Field Study was carried out in the Val d'Or region of Quebec in 1995 to evaluate a large number of environmental monitoring methods and to reduce the list of monitoring technologies for further evaluation at a cross-section of mine sites across Canada (BEAK, 1996). In 1996, a field evaluation program was initiated and involved preliminary sampling at seven candidate mine sites with the objective of identifying a short-list of mines that had suitable conditions for further detailed monitoring and testing of hypotheses relating to the AETE program. Preliminary study designs were developed for four sites that were deemed to be most suitable for hypothesis testing in 1997 (EVS *et al.*, 1997). The sites selected were Heath Steele, New Brunswick; Lupin, N.W.T.; Dome mine, Ontario; and Westmin Resources (now Boliden-Westmin), British Columbia. Lupin was subsequently dropped based on a 1997 reconnaissance survey and replaced with the Mattabi Mines Ltd. site. This report documents the results of the 1997 Field Evaluation at the Heath Steele mine site in New Brunswick.

The 1996 Field Evaluation Program constituted Phase I of the Field Evaluation Program. The 1997 program consists of Phases II and III of the Program. Phase II includes the review of necessary background information, finalization of a study design and implementation of the field studies. Phase III includes the compilation, interpretation and reporting of results.

## 1.1 Study Objectives

The overall goal of the AETE program is to identify cost-effective methods and technologies that are suitable for assessing aquatic environmental effects caused by mining activity. An effect is defined as "a measurable difference in an environmental variable (chemical, physical or biological) between a point downstream (or exposed to mining) in the receiving environment and an adequate reference point (either spatial or temporal)". For the formulation of hypotheses, this definition has been refined by the AETE Committee to distinguish between effects or responses as measured in biological variables as opposed to effects reflected in physical or chemical changes.

The questions used in developing the hypotheses to be tested in this program were:

1. Are contaminants getting into the system (and to what degree, and in which compartments)? This question relates to the presence and concentrations of metals in environmental media (e.g., water, sediments), and requires an understanding of metal dispersal mechanisms, chemical reactions in sediment and water, and aquatic habitat features which influence exposure of biological communities.

2. Are contaminants bioavailable? This question relates to the presence of metals in biota or to indicators of metal bioaccumulation such as the induction of metallothionein (a biochemical response to metal exposure) in fish tissues. Only if contaminants are bioavailable can a biological effect from chemical contaminants occur.

3. Is there a measurable response? Biological responses may occur only if contaminants are entering the environment and occur in bioavailable forms. These responses may occur at various levels of biological organization, including sub-organism levels (e.g., histopathological effects), at the organism level (e.g., as

measured in toxicity testing), or at population and community levels (as measured in resident benthos and fish communities).

4. Are contaminants causing the responses? This question is difficult to measure in field studies directly, as cause-effect mechanisms are difficult to assess under variable conditions prevailing in nature. However, correlations between measures of exposure, chemical bioavailability and response may be used to develop evidence useful in evaluating this question.

The AETE Technical Committee developed a study framework, using the above questions and the three components (water and sediment monitoring, biological monitoring in receiving waters and toxicity testing). The following eight areas of work were identified to finalize the work plan, develop the hypotheses, prioritize issues and identify field work requirements:

1. Chemical presence;
2. The overlap between communities and chemistry testing to determine whether biological responses are related to a chemical presence (bioavailability of contaminants);
3. Biological response in the laboratory;
4. Biological response in the field;
5. Chemical characteristics of the water and sediments used to predict biological responses in the field (contaminants causing a response);
6. The overlap between biological community responses and bioassay responses to evaluate whether wild community changes are predicted by bioassay responses;
7. The overlap between chemistry and bioassay responses to evaluate whether chemicals are responsible for bioassay responses; and
8. The overlap between the chemical, the exposure and the effects in the laboratory and the effects in the field.

The core objective of the 1997 field program is to **test the 13 hypotheses, developed by the AETE Committee, at as many mine sites as possible (Table 1.1)** The hypotheses are more specific questions about the ability or relative ability of different monitoring tools to answer the four general questions (above) about mine effects.

These 13 hypotheses can be categorized into:

- ***Sediment Monitoring***: evaluation of sediment toxicity testing tools (test types) as to their relative ability to detect linkages between mine exposure and sediment toxicity (H1);

- ***Biological Monitoring (in Fish)***: evaluation of tissue biomonitoring tools (measurement types) as to their ability to detect linkages between mine exposure and tissue contamination (H2 to H4); and evaluation of population/community biomonitoring tools (measurement types) as to their ability to detect linkages between mine exposure and ecological response (H5 to H8); and

- ***Integration of Tools***: evaluation of various monitoring tools as to their relative ability to detect relationships between specific measures of mine exposure and specific biological response measures, or between sediment toxicity and benthic community response measures (H9 to H12); and evaluation of effluent toxicity testing tools (test types) as to their ability to detect relationships between effluent toxicity and population/community response measures (H13).

Due to the natural characteristics of Heath Steele area watersheds, eight (8) of the 13 hypotheses were considered testable at Heath Steele (H4, H5, H6, H7, H9, H10, H12 and H13) and are highlighted in Table 1.1.

## 1.2 Site Description

Heath Steele Division of Noranda Mining and Exploration Inc. (Heath Steele) operates a base metal mining and milling operation in north-central New Brunswick, approximately 50 km northwest of the City of Miramichi (Figure 1.1). Mine/mill operations are situated within the headwaters of the Tomogonops River, a tributary system of the Northwest Miramichi River.

The Heath Steele site has a relatively long history, with mine and mill facilities first developed in 1955-1957. Heath Steele ores are base metal sulphides, with zinc, lead, copper and silver-rich concentrates produced.

Figure 1.2 shows the study area with mine sources of contaminants. The South Branch Tomogonops River receives discharge from the tailings area, but this stream has, in recent years, become periodically acidic due to thiosalt oxidation and is high in dissolved solids

(BEAK, 1997). This has produced a relatively strong pH gradient in the South Branch Tomogonops River, especially in summer. The metal concentration gradient in the South Branch is relatively weak (small changes with distance), and fish are scarce in the reach nearest the tailings pond.

The Little South Branch Tomogonops River receives seepage and runoff from the general mine site that is not strongly acidic and the water is much softer than treated effluent. These effects occur at Heath Steele monitoring station HS-3, downstream of which no significant additional inputs occur from Heath Steele. This water is relatively rich in metals, and downstream gradients in water quality and biological conditions have been well documented (BEAK, 1997). Accordingly, the 1997 AETE field program focused on river reaches in the Little South Branch Tomogonops River and waters downstream before the confluence with the South Branch Tomogonops, where water hardness level abruptly increases.

A railway bridge at times presents a barrier to upstream migration of adult salmon. Therefore, the fish community is different below the bridge than above. Fish present above the bridge include juvenile salmon, small brook trout, white sucker and minnows, although the abundance of salmon here is influenced by the barrier. An apparently fishless zone exists immediately below the mine at HS-3, apparently due to water quality impairment.

Aquatic habitat throughout this area consists of riffles and runs, with a predominantly rock-cobble-gravel streambed. Soft sediments are rare to absent throughout most of the Tomogonops River watershed. The predominant erosional condition of the river prevents effective testing of sediment monitoring tools at Heath Steele. The watershed is undeveloped and forested except for the mine site itself. The streamflow was low ($\leq 0.31$ $m^3/s$) at all locations sampled in August 1997, with typical stream widths of up to about 8 m. Stream size is progressively smaller towards upper reaches of the watershed. All reference areas selected for study herein, including the neighbouring Little River and unaffected reaches of the Tomogonops River, are similar to those represented by the area of downstream habitat sampled, except for the partial barrier noted above. Habitat information is detailed in Appendix 2.

# 2.0 STUDY DESIGN

## 2.1 Adjustments to Preliminary Study Design

The preliminary study design developed by EVS *et al.* (1997) for Heath Steele was reviewed and discussed with the AETE Technical Committee. Various important recommendations arose from this review. These recommendations received AETE's approval, and are integral to the final study design outlined in this section. Those recommendations are:

- The locations for testing of fish community response tools were relocated to the gradient beginning at HS-3 on the Little South Branch Tomogonops River, down to a point upstream of the South Branch Tomogonops confluence. This relocation stems from concerns over potential thiosalt-induced pH effects and variable water hardness effects in the South Branch (where EVS *et al.* proposed sampling), confounding the measurement of metal-induced biological effects.

- Sediment chemistry and sediment toxicity measurements were not made due to the relative lack of sediment-induced biological impacts seen at Heath Steele previously. However, periphyton is used as a surrogate sediment in testing H10.

- "Effluent" toxicity was measured at Heath Steele Station HS-3 rather than in final treated effluent, with re-focusing of seasonal sampling to dry/wet weather sampling based on suspected effects of rainfall. This is used for testing of H13 using fish and benthic data.

- Fish community/population tools have been tested, with sentinel species including Atlantic salmon juveniles and blacknose dace. Use of fully enclosed electrofishing stations to sample fish for testing of H5 have been replaced with electrofishing without block nets to allow cost-effective sampling of more stations/areas than provided in the original design (EVS *et al.*, 1997) without impairing our ability to collect meaningful catch-per-unit-effort (CPUE) measurements.

- H4 and H12 have been tested with both wild salmon juveniles and caged salmon juveniles to better determine the relative effectiveness of the metallothionein (MT) and tissue metal measurements. Caged fish were used to control fish exposure so

that fish mobility would not affect recent metal exposure and tissue response. Use of fish in cages is not considered here specifically as a monitoring tool *per se*.

## 2.2 Final Study Design

### 2.2.1 General Considerations

In general, sampling at AETE field study sites has been carried out in relation to a mine effluent discharge in order to permit testing of hypotheses about the environmental effect of the discharge. Sampling was completed both above and below the source (Reference versus Exposed). To the extent possible, the "below discharge" samples were spaced at increasing distances, because most dilution/mixing models are exponential decay models. That is, contaminant concentrations usually decrease rapidly with distance at first, and increasingly more slowly in an exponential fashion (see Figure 2.1). When monitoring mine discharges, the nature of the receiving stream will often cause this ideal situation to be impossible to achieve, especially where dilution occurs rapidly (e.g., a stream discharging into a large lake).

There are many possible field study designs for monitoring of mining discharges and testing of the hypotheses, which can be put into three basic categories (Figure 2.2, Types A, B, C). The difference between the first two (Type A versus Type B) is driven by site differences (e.g., stepwise (Type A) versus more continuous dilution patterns (Type B)), whereas the difference between Type B and Type C is driven by the biota being sampled. For example, benthos because of their sessile nature, and some forage fish because of their limited mobility, allow for replicate sampling in a small area (Type B) whereas large fish being more mobile have to be sampled over a larger area to ensure the groups of fish are not mixing and are distinct from one another, necessitating a Type C design. Alternatively, a Type A design might be used for large fish, using individual fish rather than stations as replicates.

In a way, the ideal situation for testing hypotheses for the 1997 field evaluation is a Type B study design which is a combination of easy-to-sample biota and a site which can be sampled with a gradient design approximating that described above. This provides for:

- a gradient design, permitting regression/correlation analysis of the impact pattern along the stream below the discharge and of possible cause-effect relationships between chemical and biological variables; and

- replication at locations so hypothesis testing in an Analysis of Variance (ANOVA) design is possible.

The other two types of study design sacrifice one or the other of these. In the first, the nature of the site precludes a gradient design. One takes replicate samples at an above"="Control" location, and at a "near-field"="High Impact" and at a "far-field"="Low Impact" location. This does not allow one to model the pattern of impact below the discharge, but an ANOVA for testing impact-related hypotheses is easily done. In the third type of study design, one can model the pattern of impact below the discharge but the only possible hypothesis testing is that associated with simple regression/ correlation analysis. The least desirable situation (not shown) would be a site where neither a gradient design nor replication at locations is possible.

Finally, it is necessary to select an appropriate sampling effort and (apart from the above "basic types of design" considerations) allocate the effort appropriately to above versus below the discharge areas, to locations within areas, and to replicates within locations. For the AETE program, it was decided that a total sampling effort per site of 20 to 25 field samples was a reasonable trade-off between feasibility and cost on the one hand, and statistical power and robustness on the other hand (refer to EVS *et al.*, 1997). The following is based on that total effort allocated to Heath Steele.

It should be emphasized here that the primary purpose of the 1997 field programs is to evaluate monitoring tools as to their ability to detect mine effects. This requires designing to detect effects. However, the approaches and sampling effort used here are not necessarily the same as would be required in undertaking an environmental effects monitoring (EEM) program at a mine.

### 2.2.2 *Design at Heath Steele*

**Sampling Areas**

The study design at Heath Steele is of the second type in Figure 2.2 (Type B). This was considered feasible based on a reasonably continuous pattern of dilution downstream from the mine site (Figures 2.3 and 2.4; BEAK and Golder, 1997). There are relatively homogeneous reaches of several kilometres between the tributaries that provide dilution in the Little South Branch and North Tomogonops Rivers. Therefore, it was possible to locate two stations in each of the five exposed reaches, and in each of three reference reaches (Figure 2.3), such that stations within a reach have similar effluent exposure levels.

The design is based on sampling downstream of HS-3 on the Little South Branch Tomogonops River (labelled "ARD Effluent' in Figure 2.3). This is recognized as the location most affected by acid rock drainage (ARD) from Heath Steele. Most of the total loadings of important metals (Zn, Cu) from Heath Steele occur in this vicinity rather than from the tailings pond, which discharges treated effluent to the South Branch Tomogonops River.

Five exposure areas were sampled downstream of the ARD effluent, corresponding with average effluent concentrations of 60% (at HE1 located on Little South Branch) to 12% at HE5 (downstream of Island Lake Brook) (Figure 2.3). Exposure Areas 1 to 4 (HE1 to HE4) are influenced by the partial barrier to salmon migration located downstream of HE4 at the railway crossing; therefore, HE5 is not comparable to upstream areas in terms of salmon CPUE measurements, but is comparable in this respect to HR3. Only Reference Area HR2 is comparable to Exposure Areas HE1 to HE4 in terms of these factors. All reference areas were used for testing of fish tissue and fish population/community level hypotheses responses.

The two sentinel fish species sampled were blacknose dace and juvenile Atlantic salmon. Among the fish species present, Atlantic salmon juveniles are generally the most ubiquitous and abundant in the exposure zone, with the exception that this species does not appear to enter the Little South Branch Tomogonops River (LSBTR). Nearly all fish present in the river are small (typically ≤12 cm in fork length), and are not amenable to contaminant analysis of individual tissues.

Hypothesis H4 was tested at Heath Steele not only with blacknose dace and wild juvenile salmon, but also using caged Atlantic salmon installed at two stations in each of the five exposure reaches and three reference reaches. These salmon were taken from the Heath Steele salmon rearing facility, located upstream of any known sources of metal loadings from Heath Steele. Some data are also available for brook trout at Heath Steele, but the data were not subjected to hypothesis testing.

Benthic-related hypotheses were tested at Heath Steele in all exposure and reference areas. All areas contain cobble/gravel substrates, and reference areas span a range of stream size conditions from HR1, which is similar in stream size to the LSBTR at HE1, through to Reference Areas HR2 and HR3 which are more comparable to the middle and lower Exposure Areas (HE3 to HE5).

### 2.2.3 Statistical Power

The statistical power of the study design was evaluated using the Borenstein and Cohen (1988) computer code for power analysis. The total effort of 16 sampling stations equally distributed among 8 groups (stream reaches) is sufficient to expect that an effect size (average difference between groups) of three within-group standard deviations could be detected with a power of 0.8 or better (i.e., chance of false-negative conclusion (beta) less than 0.2) using a significance criterion based on a chance of false-positive conclusion (alpha) less than 0.05. The absolute difference indicated by three standard deviations will vary from one monitoring parameter (effect measure) to another.

Sheet1

| Predicted Effluent Concentrations at Heath Steele Stations | |
|---|---|
| Station I.D. | Concentration |
| HS-3 | 1 |
| HE1 | 0.6 |
| HE2 | 0.49 |
| HE3 | 0.15 |
| HE4 | 0.13 |
| HE5 | 0.12 |

**Figure 2.4: Relative Concentration of HS-3 "Effluent" at Heath Steele Exposure Reaches, Based on 20 August 1997 Stream Flows**

# 3.0 FIELD AND LABORATORY METHODS

## 3.1 Sampling Time and Crew

The Heath Steele field program was carried out during the period of 11 to 22 August 1997.

The field crew consisted of two field biologists and two technicians. The BEAK project manager also participated during half of the program.

## 3.2 Sampling Effort and Station Characterization

The numbers and distributions of each type of sample collected at Heath Steele are summarized in Table 3.1. Variable numbers of fish tissues collected at each station reflect the presence, absence and abundances of various species.

Sampling stations for the Heath Steele program are listed in Section 2.2.2 and illustrated in Figure 2.3. These include five "reaches" (HE1 to HE5) downstream of the "effluent" source (HS-3) and three reference "reaches" (HR1 to HR3). Each downstream reach contained two stations (A and B) of similar effluent concentration, separated by several hundreds of metres. All stations were sited at least 150 m from major stream confluences to avoid exposure to uneven effluent concentrations and to provide some assurance that biological communities sampled were generally resident under site conditions (i.e., had not recently migrated from other streams of different water quality).

Habitat conditions and station coordinates, measured by Global Positioning System, were recorded on data forms (Appendix 2). Habitat information included stream order, data on water temperature, conductivity, pH, substrate conditions, pool/riffle ratio, aquatic plant coverage, in-stream and riparian cover, water depth and general flow conditions. All stations may be generally characterized as riffle-run sequences, with cobble and gravel substrates.

Habitat conditions are affected by barriers to fish migration including an abandoned railway crossing between exposure reaches HE4 and HE5, which presents a partial barrier for salmon migration (long culverts terminating about 0.5 metres above river level on the downstream side), and two Heath Steele reservoirs on the Little South Branch Tomogonops between exposure reach HE1 and reference reach HR1 which preclude migration of Atlantic salmon to the HR1 area. For this reason, salmon abundance in exposure reaches HE1 to HE4 can only be compared with

TABLE 3.1: SUMMARY OF SAMPLES OBTAINED AT HEATH STEELE

| Sampling Locations | Chronic Toxicity[1] | Periphyton and Benthos[2] | Water Quality[3] | Fish Tissues for Analysis[4] | | | Fish Community[6] |
|---|---|---|---|---|---|---|---|
| | | | | ASW | BD[5] | ASC | |
| HS-3 | 3 | - | 3 | - | - | - | - |
| HE1A | - | 1 | 1 | 0 | 0 | 2 | 1 |
| HE1B | - | 1 | 1 | 0 | 1 (1) | 2 | 1 |
| HE2A | - | 1 | 1 | 0 | 0 | 2 | 1 |
| HE2B | - | 1 | 1 | 1 | 4 (14) | 2 | 1 |
| HE3A | - | 1 | 1 | 3 | 1 (5) | 2 | 1 |
| HE3B | - | 1 | 1 | 4 | 1 (6) | 2 | 1 |
| HE4A | - | 1 | 1 | 4 | 0 | 2 | 1 |
| HE4B | - | 1 | 1 | 4 | 4 (13) | 2 | 1 |
| HE5A | - | 1 | 1 | 4 | 5 (16) | 2 | 1 |
| HE5B | - | 1 | 1 | 4 | 6 (16) | 2 | 1 |
| HR1A | - | 1 | 1 | 0 | 4 (12) | 2 | 1 |
| HR1B | - | 1 | 1 | 0 | 4 (15) | 2 | 1 |
| HR2A | - | 1 | 1 | 0 | 6 (16) | 2 | 1 |
| HR2B | - | 1 | 1 | 1 | 4 (16) | 2 | 1 |
| HR3A | - | 1 | 1 | 4 | 3 (7) | 2 | 1 |
| HR3B | - | 1 | 1 | 4 | 2 (9) | 2 | 1 |

[1] Chronic toxicity samples collected 24 June, 28 August and 12 November 1997.

[2] Each periphyton sample is a composite of scrapings from ≥3 rocks. Each T-sample is a composite of five grabs.

[3] Water quality samples, exclusive of blanks, duplicates.

[4] ASW - wild Atlantic salmon parr; BD - blacknose dace; ASC - caged Atlantic salmon.

[5] BD - several fish submitted per sample for tissue analysis to allow for compositing at laboratory to meet sample mass requirements. Variable numbers of composite BD samples analyzed per station. Values represent numbers of composite samples, with total numbers of individual fish in all composites combined in parentheses.

[6] Community sample based on approximately 1,000 to 1,900 measured electrofishing seconds per station. All fish identified, enumerated, weighed and measured (length). Sentinel species caged by length-frequency distribution with ages determined by scale (BD) or otolith (AS) to confirm age-size class categories.

abundances at reference station HR2. Salmon abundance at HE5, which is unaffected by downstream migration barriers, may be compared with abundance at reference reach HR3.

At selected stations (generally one in each reach), in-stream discharge was measured using the cross-section of method with a portable velocity meter (Marsh McBirney, Model No. 2000-11). All discharge measurements were taken under dry weather conditions (no precipitation during the previous 48 hours) on 20 August 1997, so that discharges at each reach would be proportional to one another. Discharge at HS-3 on the same date was provided by Heath Steele, as recorded at their stream gauge. Because "effluent" discharge rates are controlled mainly by natural drainage processes, effluent dilution factors within each reach are approximately constant. "Best estimates" of streamflow were made by considering not only the measurements made, but also the suitability of each streamflow measurement location for providing accurate discharge estimates (e.g., degree of turbulence), the watershed area of each reach and the concentrations of suitable effluent tracers such as total zinc concentration. These final best "estimates" are those used to produce the relative effluent concentrations for each reach (Figure 2.4), and are presented in Appendix 2.

## 3.3 Effluent Chemistry and Toxicity

Chronic toxicity was measured in three samples of HS-3 "effluent" from Heath Steele, collected on 24 June, 28 August and 12 November 1997. The August sample was collected during a runoff event in an attempt to sample a more metal-rich effluent normally found during higher flow conditions at this location. Tests completed on each sample include:

- the *Ceriodaphnia dubia* 7-day survival and reproduction test (Environment Canada 1992a)
- the fathead minnow (*Pimephales promelas*) 7-day survival and growth test (Environment Canada 1992b)
- the *Selenastrum capricornutum* 3-day algal growth test, (Environment Canada 1992c), and
- the duckweed (*Lemna minor*) 7-day growth test (Saskatchewan Research Council, 1995, 1996).

The duckweed test was carried out by the Saskatchewan Research Council, in Saskatoon. The other three tests were completed at BEAK's Brampton, Ontario toxicity testing facility. Toxicity testing procedures and laboratory reports are presented in Annex 1.

Bioassay procedures included use of dilution water collected from the site (Little South Branch Tomogonops upstream of mine-related impact) or laboratory water adjusted to the hardness of field conditions, depending on acclimation success in site water for *Ceriodaphnia dubia* and *Pimephales promelas*. Results of a comparative study of chronic toxicity using both site dilution water and hardness adjusted laboratory water, in addition to acclimated organisms and organisms not acclimated, are presented in a Summary Document for the three mines where effluent toxicity was measured in the 1997 AETE field study program (BEAK and GOLDER, 1998b). Results of this comparative study showed that site dilution water and laboratory dilution water produced generally comparable results in these tests.

Upon receipt at BEAK's laboratory, a subsample of each effluent and dilution water sample was forwarded to Philip Analytical Services. Samples were processed (filtered as appropriate and preserved) and analyzed for the water quality parameters identified in Section 3.4.

## 3.4 Water Quality

Detailed field sampling procedures, including water quality sampling procedures, are outlined in Annex 1 (provided as a separate document).

### 3.4.1 Field

All water samples were collected on 20 August 1997 under dry weather conditions (no precipitation over previous 48 hours) so that relative metal concentrations at all locations were representative of the same effluent quality (water quality at HS-3 effluent varies according to runoff). Samples were collected for laboratory analysis of:

- total and dissolved metals (Al, Sb, As, Ba, Be, Bi, B, Cd, Ca, Cr, Co, Cu, Fe, Pb, Mg, Mn, Hg, Mo, Ni, K, Se, Ag, Sr, Ta, Sn, U, V, B and Zn); Zn, Cu, Pb, Cd, Fe and Al are most relevant at Health Steele, based on effluent concentrations observed;
- nutrients (nitrate, nitrite, ammonia, P);
- major ions (including sulphate and ion balance);
- acidity, alkalinity, hardness, specific conductance;
- pH;
- colour;
- dissolved organic and inorganic carbon;

**Table 3.2: LABORATORY METHODS AND BOTTLE/PRESERVATIVE PROCEDURES USED IN WATER SAMPLE ANALYSIS**

( as provided by Philip Analytical Services)

| Parameters | Method | Bottle Requirement | Preservative Type | Max. Holding Time |
|---|---|---|---|---|
| **Acidity** | Standard Methods (17th ed.) No. 2310B<br>U.S. EPA Method No. 305.1 | 250 ml Bottle Glass | no preservative | 14 days |
| **Alkalinity** | Standard Methods (17th ed.) No. 2320 | 250 ml Bottle Glass | no preservative | 14 days |
| **RCAP Calculations**<br>Total Dissolved Solids(Calculated)<br>Hardness(as $CaCO_3$)<br>Bicarbonate(as $CaCO_3$, calculated)<br>Carbonate(as $CaCO_3$, calculated)<br>Cation Sum<br>Anion Sum<br>Ion Balance | MDS Internal Reference Method | | | |
| **Colour** | U.S. EPA Method No. 110.3(Modified)<br>(Reference-Std Methods(17th)2120CMod) | 100 ml Bottle Glass | no preservative | 48 hours |
| **Specific Conductance** | U.S EPA Method No. 120.1 | 100 ml Bottle Glass | no preservative | 28 days |
| **Manual Conventionals for RCP(pH,Turb,Conduct,Color)** | U.S. EPA Method No. 150.1, 120.1, 180.1<br>and 110.3 | 250 ml Bottle HDPE | no preservative | |
| **Hardness** | U.S. EPA Method No. 130.2 | 250 ml Bottle Glass | no preservative | 6 months |
| **Ion Balance** | | 250 ml Bottle HDPE | $HNO_3$ to pH < 2 | 14 days |
| **pH, Hydrogen Ion Activity** | U.S. EPA Method No. 150.1 | 100 ml Bottle Glass | no preservative | |
| **Total dissolved Solids** | U.S. EPA Method No. 160.1 | 1 L Bottle Glass | no preservative | 7 days |
| **Total Suspended Solids** | U.S. EPA Method No. 160.2 | 500 ml Bottle Glass | no preservative | 7 days |
| **Turbidity, UltraViolet** | U.S. EPA Method No. 180.1 | 100 ml Bottle Glass | no preservative | 48 hours |
| **RCAP MS Package, 8 Element ICPAES Scan**<br>B, Fe, P, Zn, Ca, Mg, K, Na | U.S. EPA Method No. 200.7 | 125 ml Bottle HDPE<br>250 ml Bottle HDPE | $HNO_3$ to pH < 2<br>no preservative | |
| **ICP-MS 25 Element Scan, Clean Water Package**<br>Al, Sb, As, Ba, Be, Bi, Cd, Cr, Co, Cu, Pb, Mn, Mo, Ni, Se,<br>As, Sr, Th, Sn, Ti, U, V, B, Fe, Zn | U.S. EPA Method No. 200.8(Modification) | 250 ml Bottle HDPE<br>125 ml Bottle HDPE | no preservative<br>$HNO_3$ to pH < 2 | |
| **Alkalinity for RCAP Packages 30, 50 and MS** | U.S. EPA Method No. 310.2 | 250 ml Bottle HDPE | no preservative | 14 days |
| **Anions for RCAP 50 and MS($Cl$,$NO_2$,$NO_3$,o-$PO_4$ & $SO_4$)** | U.S. EPA Method No. 300.0 or<br>U.S. EPA Method No. 350.1, 354.1, 353.1,<br>365.1 and 375.4. | 250 ml Bottle HDPE | no preservative | 48 hours |
| **Dissolved Organic Carbon, as Carbon for RCAP** | MOE Method No. ROM - 102ACE(Modified) | 100 ml Bottle Glass | no preservative | 3 days |
| **Ammonia for RCAP Packages 30, 50 and MS** | ASTM Method No. D1426-79 C<br>Refer - Method No. 1100106 Issue 122289 | 100 ml Bottle Glass<br>250 ml Bottle HDPE | $H_2SO_4$ to pH < 2<br>no preservative | 28 days |
| **Organic Nitrogen(TKN - $NH_3$)** | U.S. EPA Method No. 350.1<br>U.S. EPA Method No. 351.1 | 250 ml Bottle Glass | $H_2SO_4$ to pH < 2 | 28 days |
| **Mercury, Cold Vapour AA** | U.S. EPA SW846 Method No. 7470A<br>Standard Methods(18th ed.) No. 3112B | 100 ml Bottle Glass | $HNO_3$ to pH < 2<br>+ 5% $K_2Cr_2O_7$ | 7 days |

- solids (total suspended and dissolved); and
- turbidity.

In addition to samples collected for laboratory analysis, field determinations were made of specific conductance, temperature, pH and dissolved oxygen, with results recorded on field habitat record forms. All field measurements were made on-site using calibrated meters.

All samples were placed on ice in coolers immediately after collection, and were transferred to a refrigerator prior to field processing. All samples requiring analysis without chemical preservation were kept chilled until delivery to the laboratory.

Sample containers, filtration and sample preservation procedures are identified in Annex 1, and include use of high density polyethylene containers confirmed free of measurable metal contamination, ultrapure nitric acid and de-ionized distilled water (for field, trip and filter blanks), and a filtration procedure using polypropylene syringes with 0.45 micron syringe-filters confirmed free of measurable metal contamination. All sample preparation was carried out in a clean work space in a hotel unit.

Quality control/quality assurance procedures followed in the field included collection of sample duplicates, and preparation of trip blanks, field blanks and filter blanks.

### 3.4.2 Laboratory

All water samples were forwarded to the analytical laboratory (Philip Analytical Services Corporation, Burlington and Mississauga, Ontario) within 48 hours of collection. Procedures used for laboratory analysis are summarized in Table 3.2.

## 3.5 Periphyton

Periphyton was collected for metals and taxonomic analysis at each of the 16 Heath Steele stations. One sample each for taxonomic and metal determination was collected at each location (i.e., total of two samples per reach).

Collections for taxonomic evaluation were made by manually scraping surfaces of three rocks on the stream bottom using a stainless spatula approximating the method of Rott (1995). The areas were scraped inside a 1 $cm^2$ measured area, with a minimum of 3 $cm^2$ sampled (1 $cm^2$ per rock), or until about 1 mL (wet volume) of material was obtained. Sample areas were recorded and the samples diluted to about 10 mL with site water. Periphyton samples for taxonomic analysis were then preserved with Lugol's iodine.

Taxonomic determinations were completed in the laboratory of Dr. H.C. Duthie, Department of Biology, University of Waterloo. These determinations include species identifications and biomass of each.

Samples for metal analysis in periphyton were collected in a similar fashion without measurement of sample area. Samples were scraped from the same three rocks sampled for taxonomy or, where periphyton growth was very light, also from neighbouring rocks. Samples were scraped until a wet volume of about 1 mL was reached, and were placed in small high density polyethylene bottles. Samples were then preserved by freezing until delivery to Philip Analytical Laboratories for metals analysis. Samples were analyzed by ICP-Mass Spectroscopy after drying and digesting the sample.

Quality control/quality assurance procedures included collection of duplicate samples for metal analysis.

## 3.6 Benthic Macroinvertebrates

### 3.6.1 Field

One benthic sample was collected at each of the two stations within each exposure and reference reach. Each sample consisted of a 5-grab composite using a 0.1 $m^2$ T-sampler fitted with a 250 micron mesh collection net. Samples were collected by manually removing invertebrates from

rock surfaces and disturbing the underlying sand and gravel repetitively to a depth of about 10 cm. All collections were made by the same field crew member.

After collection, each composite sample was preserved in a clearly labelled 1 L plastic jar and preserved to a level of 10% buffered formalin.

### 3.6.2 Lab Processing

All samples were processed by the BEAK Benthic Ecology Laboratory or by Zaranko Environmental Assessment Services, Guelph, Ontario. Both laboratories followed the same laboratory protocols.

In the laboratory, samples were inspected to insure that they were adequately preserved and correctly labelled. Samples were then stained to improve sorting recovery.

Prior to detailed sorting, the samples were washed free of formalin in a 250 µm sieve under ventilated conditions. The benthic fauna and associated debris were then elutriated free of any sand and gravel. The remaining sand and gravel fraction was closely inspected for any of the denser organisms, such as Pelecypoda, Gastropoda, and Trichoptera with stone cases that may not have all been washed from this fraction. The remaining debris and benthic fauna after elutriation were washed through 500 µm and 250 µm sieves to standardize the size of the debris being sorted and facilitate a minimum of 95% recovery of benthic fauna.

All benthic samples were processed with the aid of stereomicroscopes. A magnification of at least 10X was used for macrobenthos (invertebrates >500 µm) and 20X for meioinvertebrates (invertebrate size >250 to <500 µm). Benthos was sorted from the debris, enumerated into the major taxonomic groups, usually order and family levels and placed in vials for more detailed taxonomic analysis.

Benthic invertebrates were most commonly identified to the lowest practical level, genus or species for most groups. The level to which each group was identified and the taxonomic keys that the identification were based on are provided in Annex 1.

Benthic samples, especially when consisting of composites of multiple samples, often require extensive hours and costs for sorting. In addition, technicians working for extended periods on one sample often become fatigued and sorting efficiency and accuracy reduce significantly.

Samples either need to be subsampled because of large amounts of organic matter or due to high densities of invertebrates. The latter is the case at Heath Steele.

For Heath Steele samples, a minimum of 400 to 600 organisms was sorted from each sample. Subsampling was based on the weight of the sample. Each whole sample was drained of water, homogenized, and sample portions randomly selected until a prescribed weight of material was attained. For example, if the total sample weighed 5,000 g, typically 25%, or 1,250 g would be selected for sorting. For Heath Steele, subsample fractions as low as 5% were sufficient to obtain more than 500 animals, based on five pooled samples.

Subsampling error was determined for both density and number of taxa in 10% of the samples that were subsampled. Ten percent of sorted samples were resorted by an independent taxonomist to ensure 95% recovery of all invertebrates. At least 95% recovery of organisms is required to meet BEAK's data quality objective.

A voucher collection or reference collection of benthic invertebrate specimens was compiled for Heath Steele. This is a collection of representative specimens for each taxon so that there can be continuity in taxonomic identifications if different taxonomists process future samples. The voucher collection will be maintained at BEAK. The BEAK Benthic Ecology Laboratory also maintains a master reference collection of all taxa which have been identified by the lab.

The specimens selected for the voucher collection were preserved such that they will remain intact for many years. Chironomids and oligochaetes remain on the initial slides and representatives of each taxon were circled with a permanent marker and labelled. All other species were preserved in 80% ethanol in separately labelled vials. Each vial contains a 3% solution of glycerol to prevent spoilage of the fauna if the vials accidentally dry out.

### 3.6.3 Chironomid Deformities

In the last decade there has been considerable attention paid towards the use of chironomid mouth part deformities to monitor contaminant effects. Previous studies have shown that the incidence of chironomid deformities (especially in *Chironomus*) can be associated with contaminated sediments.

For the 1997 study, all mounted chironomid specimens from each site were scored for mandible and mentum deformities or abnormalities. These data were not used in the testing of specific hypotheses, but are discussed briefly in Section 4.4.

## 3.7 Fish

### 3.7.1 Wild Fish Collections

Wild fish were sampled at each of the 16 stations (2 stations per reach, HE1 to HE5, HR1 to HR3) during the August 1997 field survey. Sampling was carried out using a portable back-pack electrofishing unit (Smith Root Model XV). Sampling was carried out in the same vicinity as benthic collections, with care taken to avoid disruption of benthic substrates by placement of the electrofishing area at least 50 m downstream of benthic sampling sites.

Electrofishing was carried out with a standardized effort of approximately 15 to 20 electrofishing minutes (as lapsed on the electrofisher counting unit), or about one hour of actual time. The crew consisted of one electrofisher operator using an anode equipped with a capture net, and a technician using a long-handled dip net to assist in fish collection. Effort (shocking seconds) was recorded for each station. Stations were not enclosed with block nets, and all habitat conditions represented at each site were sampled.

All captured fish were retained in a 20-L plastic bucket containing site water until completion of sampling. After collection, all fish were identified and weighed on-site, and were either retained for further analysis (frozen whole on dry ice for metallothionein or metal analysis, or for determination of age and organ size) or released back to the river. Fish lengths were measured using standard measuring boards (total length, fork length) to the nearest millimetre. Weights to the nearest 0.1 g were determined using an Ohaus balance. A more detailed account of procedures used in processing of fish samples is presented in Annex 1.

The two sentinel species retained for tissue analysis were juvenile Atlantic salmon parr and adult blacknose dace. Wherever possible, sufficient numbers were retained for a minimum of two samples per species for each of metallothionein (MT) and metals in viscera. One to four (usually four) wild juvenile salmon per site and up to 16 blacknose dace per site were retained for this purpose (blacknose dace required composites of more than one fish to produce adequate visceral mass for laboratory analyses). As potential alternate sentinel species,

several lake chub and/or small brook trout were also retained frozen from stations where these species were obtained.

Upon completion of the wild fish survey, an evaluation was made of the numbers and biomass of sentinel species (and alternates) captured at each site. Where the numbers of fish available for tissue analysis appeared deficient, supplemental electrofishing was carried out at sampling stations with effort focused on habitats most likely to produce additional specimens. These additional fish were excluded from analysis of fish community characteristics (i.e., catch-per-unit-effort CPUE).

Biological measurements carried out on sentinel species at the laboratory included age determination. Age was determined for both sentinel species by length-frequency distributions with reaches (where adequate numbers were obtained) or within reference versus exposure areas, with multi-modal distributions used to distinguish age classes. Representative specimens of blacknose dace were aged by scale reading and of Atlantic salmon by otolith to confirm age breaks implied in the length frequencies. Except for salmon fry which are easily distinguished in the field, only those fish directly aged (i.e., by scale or otolith readings) were used in the assessment of fish growth.

An attempt was made in the laboratory to measure liver weights in blacknose dace. However, after thawing, livers in the fish fragmented easily when dissected, and it was not possible to obtain all of the liver mass in each case. The fact that blacknose dace livers are diffusely distributed through the gut made effective removal more difficult. Accordingly, no liver weight determinations were recorded.

Atlantic salmon is a species of considerable resource value in the Northwest Miramichi River watershed. Thus, few juvenile salmon were retained for age determination by otolith, and larger sample sizes were not retained for organ size determinations. Only specimens for MT and metal analysis and a few others were aged directly by otolith.

### 3.7.2 Caged Atlantic Salmon

Caged Atlantic salmon juveniles were used to further evaluate the tissue metal and MT tools. The source of salmon used here was the Heath Steele McCormack Reservoir salmon rearing facility. (The McCormack Reservoir is located upstream of any significant metal sources from Heath Steele.) All fish used were yearling parr (1+).

Fish cages consisted of 20-L plastic buckets, fitted with "snap-on" plastic lids. Buckets each contained three large surface area openings covered with 1 mm "Nitex" screen. Approximately one-third of each bucket consisted of window, so that once immersed in the river, the river current would flow through the bucket.

One fish cage containing five salmon parr was installed at each of the 16 monitoring stations. Cages were placed in areas of gentle current to ensure continuous flow of water through the interior, and were secured by rope to trees or shrubs on the streambank. Fish were left in place for nine days during the August 1997 field campaign.

At the end of the exposure period, fish survival was recorded (all fish survived at all locations) and two specimens were sacrificed for metal and MT analysis of the viscera. Specimens for analysis were measured (total and fork length), weighed to the nearest 0.1 g and placed whole on dry ice. No samples of pre-exposure fish were collected for analysis as it was unnecessary in the context of hypothesis testing. However, pre-exposure fish analyzed in the fish cage experiment at Dome under the 1997 AETE program did show that tissue concentrations of metals and MT may change in response to the caging itself (refer to BEAK, 1998a).

### 3.7.3 Tissue Metallothionein and Metal Analyses

All analyses of Heath Steele fish tissues were carried out at the Department of Fisheries and Oceans, Freshwater Institute, Winnipeg, under the direction of Dr. J. Klaverkamp. Analyses were completed on two wild Atlantic salmon samples, two blacknose dace samples and two caged Atlantic salmon samples for all stations, where sufficient fish specimens were available (refer to Table 3.1). In addition, one composite gill sample was analyzed from two caged salmon per station. Variable numbers of brook trout (viscera) sampled coincidentally with the Atlantic salmon and blacknose dace were also analyzed on an opportunistic basis by Dr. Klaverkamp. The gill and brook trout data were not subject to formal hypothesis testing.

# 4.0 DATA OVERVIEW

## 4.1 Effluent Chemistry and Toxicity

Detailed toxicity test reports are presented under separate cover as Annex 1, with results summarized in Table 4.1, Figure 4.1 and Appendix 4. Effluent quality conditions are provided in Table 4.2, with laboratory reports on effluent and site dilution water quality provided in Annex 1.

All samples produced chronic toxicity in all tests except for fathead minnow. The June effluent sample was non-toxic to fathead minnow. The *Selenastrum* and *Ceriodaphnia* tests were the most sensitive of the four tests. Toxicity of the three samples to *Selenastrum* and *Ceriodaphnia* ranked in accordance with the total zinc and copper concentrations present (i.e., highest and lowest metal concentrations corresponded with the most and least toxicity), although this pattern did not hold for fathead minnow or duckweed. The duckweed response appeared to show the poorest correspondence with metal concentration, with the lowest zinc and copper concentrations producing the greatest toxicity.

The August "runoff event" sample was richer in particulate iron (i.e., total minus dissolved iron) than either of the other two dry weather samples, although both total and dissolved zinc and copper were higher in concentration in the November sample. Construction of a new buffer storage pond by Heath Steele in 1997 has apparently been successful in reducing maximum metal concentrations at HS-3 during runoff conditions, and may have contributed to a suppressed spike in metal concentrations during the August event.

## 4.2 Water Quality

Water quality data for Heath Steele are summarized in Table 4.3 (total metals and general chemistry) and Table 4.4 (which compares total versus dissolved metals). The mean concentrations for each reach are illustrated in Figure 4.2. Non-detect samples were assigned concentrations equal to half the detection limit for computation of means. Detailed data for all parameters and samples are presented in Appendix 4. These additional parameters include those that were generally below detection limits and those that did not show a mine-related trend.

As shown in Table 4.3 and graphically in Figure 4.2, total and dissolved concentrations of zinc, cadmium, lead, copper, aluminum and iron all show clear concentration gradients downstream of

**Table 4.1: Results of Aquatic Toxicity Tests Conducted on Three Heath Steele Effluent Samples (HS-3), June, August and November 1**

(Expressed as % Effluent. Values in parentheses represent the 95% confidence interval)

| Sample | *Ceriodaphnia dubia* | | | *Pimephales promelas* (Fathead Minnow) | | | *Selenastrum capricornutu* (Algae) | | *Lemna minor* (Duckweed) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | LC50 [1] | IC25 [2] | IC50 [3] | LC50 | IC25 | IC50 | IC25 | IC50 | IC25 | IC50 |
| H-E-1 (June 24-97) | 91.6 (50-infinity) | 58.4 (48.7-63.7) | 75.7 (69.7-82.3) | >100 na | >100 na | >100 na | 23 (17.9-26.0) | 55.6 (52.3-57.7) | 30.0 (17.2-52.5) | 91.1 (56.7-100) |
| H-E-2 (August 28-97 | 33.0 (28.9-37.6) | 28.4 (21.8-30.9) | 35.6 (32.8-37.5) | 22.2* (18.5-26.6) | 23.0* (16.3-34.4) | 41.0* (35.7-45.0) | 21.7 (14.6-27.5) | 32.5 (27.1-36.1) | 51.9 (45.6-59.1) | 78.4 (73.6-83.5) |
| H-E-3 November 12-9 | 18.6 (12.6-27.7) | 10.9 (4.82-18.5) | 23.0 (12.7-31.3) | 44.0* (36.9-51.4) | 41.3* not calculable | >50* na | 6.03 (4.11-11.2) | 23.7 (3.88-31.8) | 59.3 (52.5-66.9) | >100 na |

**Notes:**

All tests conducted using site water as dilution water except where indicated by "*".

* tests conducted using laboratory water (adjusted to site water hardness, pH and alkalinity) as dilution water because fish could not be acclimated to site water.

*Ceriodaphnia* and fathead minnows were acclimated to dilution water prior to testing.

Fathead minnow data analysed according to Environment Canada amendments (Nov. 1997) - IC values represent growth effects alone.

August 28 sample corresponds with runoff event.

[1] LC50 - concentration lethal to 50% of the test organisms

[2] IC25 - inhibition concentration - 25% response (i.e., 25% reduction in growth or reproduction)

[3] IC50 - inhibition concentration - 50% response (i.e., 50% reduction in growth or reproduction)

[4] not calculable by ICPIN program because random sampling of raw data resulted in an estimation of an endpoint greater than 100%.

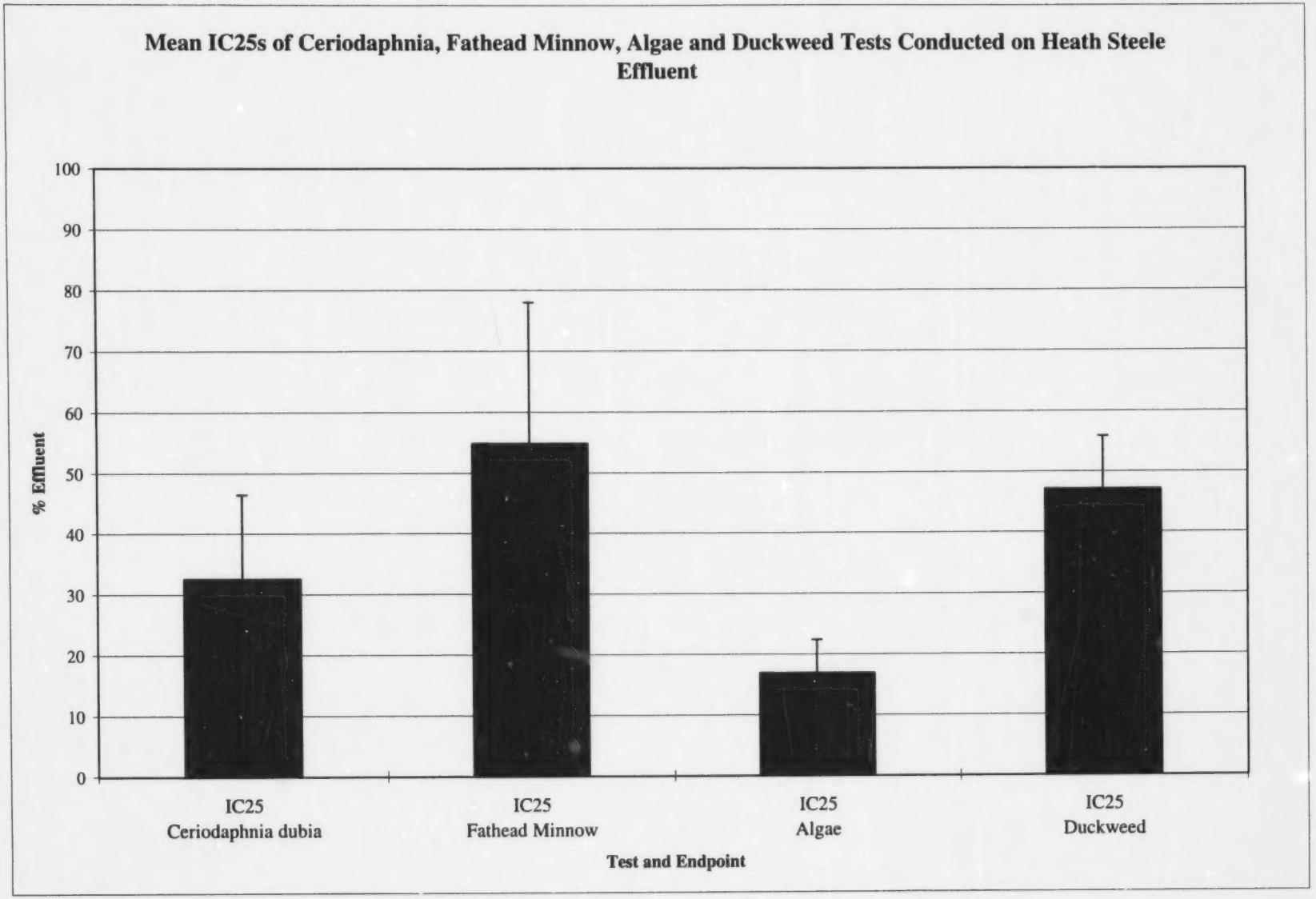

Mean IC25s of Ceriodaphnia, Fathead Minnow, Algae and Duckweed Tests Conducted on Heath Steele Effluent
100
90
80
70
60
50
40
30
20
10
0
% Effluent
IC25
Ceriodaphnia dubia
IC25
Fathead Minnow
IC25
Algae
IC25
Duckweed
Test and Endpoint

**Table 4.2: Water Quality of "Effluent" Samples (HS-3) collected at Heath Steele Mine, June, August and November 1997.**

| Parameter | Units | LOQ[1] | MMLER[2] Monthly Mean | MMLER[2] Grab Sample Maximum | HSE-1 (Total) 97/06/25 | HSE-1 (Dissolved) 97/06/25 | HSE-2 (Total) 97/08/29 | HSE-2 (Dissolved) 97/08/29 | HSE-3 (Total) 97/11/13 |
|---|---|---|---|---|---|---|---|---|---|
| Acidity(as $CaCO_3$) | mg/L | 0.1 | na[3] | na[3] | - | -[4] | - | - | - |
| Alkalinity(as $CaCO_3$) | mg/L | 1 | na | na | 5 | - | 7 | - | 1 |
| Aluminum | mg/L | 0.01/0.005 | na | na | 0.29 | 0.2 | 0.355 | 0.122 | 0.56 |
| Ammonia(as N) | mg/L | 0.05 | na | na | 0.06 | - | nd | - | nd |
| Antimony | mg/L | 0.002/0.0005 | na | na | <0.002 | <0.002 | <0.0005 | <0.0005 | <0.0005 |
| Arsenic | mg/L | 0.002 | 0.5 | 1.0 | nd | nd[5] | nd | nd | nd |
| Barium | mg/L | 0.005 | na | na | nd | nd | 0.005 | 0.005 | 0.01 |
| Beryllium | mg/L | 0.005 | na | na | nd | nd | nd | nd | nd |
| Bicarbonate(as $CaCO_3$, calculated) | mg/L | 1 | na | na | 5 | - | 7 | - | 1 |
| Bismuth | mg/L | 0.002 | na | na | nd | nd | nd | nd | nd |
| Boron | mg/L | 0.005 | na | na | 0.127 | nd | nd | nd | nd |
| Cadmium | mg/L | 0.0005 | na | na | nd | nd | 0.00067 | 0.0007 | 0.00095 |
| Calcium | mg/L | 0.1 | na | na | 3.3 | 3.7 | 4.4 | 4.4 | 4.3 |
| Carbonate(as $CaCO_3$, calculated) | mg/L | 1 | na | na | nd | - | nd | - | nd |
| Chloride | mg/L | 1 | na | na | 2 | - | 2 | - | 3 |
| Chromium | mg/L | 0.002/0.0005 | na | na | <0.002 | <0.002 | 0.0006 | <0.0005 | 0.0005 |
| Cobalt | mg/L | 0.001/0.0002 | na | na | 0.002 | 0.002 | 0.0038 | 0.0038 | 0.0078 |
| Colour | TCU | 5 | na | na | 43 | - | 79 | - | 66 |
| Conductivity - @25øC | us/cm | 1 | na | na | 42 | - | 48 | - | 56 |
| Copper | mg/L | 0.002/0.0003 | 0.3 | 0.6 | 0.023 | 0.017 | 0.0329 | 0.0262 | 0.055 |
| Dissolved Inorganic Carbon(as C) | mg/L | 0.5/0.2 | na | na | - | 1.1 | - | 0.2 | - |
| Dissolved Organic Carbon(DOC) | mg/L | 0.5 | na | na | - | 4.2 | - | 5 | - |
| Hardness(as $CaCO_3$) | mg/L | 0.1 | na | na | 13.5 | - | 16.5 | - | 15.9 |
| Iron | mg/L | 0.02 | na | na | 0.41 | 0.28 | 0.9 | 0.19 | 0.41 |
| Lead | mg/L | 0.0001 | 0.2 | 0.4 | 0.0028 | 0.0015 | 0.0048 | 0.0011 | 0.003 |
| Magnesium | mg/L | 0.1 | na | na | 1 | 1.1 | 1.2 | 1.3 | 1.2 |
| Manganese | mg/L | 0.002/0.0005 | na | na | 0.104 | 0.082 | 0.157 | 0.152 | 0.22 |
| Mercury | mg/L | 0.0001 | na | na | nd | nd | nd | nd | nd |
| Molybdenum | mg/L | 0.002/0.0001 | na | na | <0.002 | <0.002 | <0.0001 | <0.0001 | 0.0005 |
| Nickel | mg/L | 0.002/0.001 | 0.5 | 1.0 | <0.002 | <0.002 | 0.002 | 0.002 | 0.003 |
| Nitrate(as N) | mg/L | 0.05 | na | na | - | nd | - | nd | - |
| Nitrite(as N) | mg/L | 0.01 | na | na | - | nd | - | nd | - |
| Orthophosphate(as P) | mg/L | 0.01 | na | na | - | nd | - | nd | - |
| pH | Units | 0.1 | 6.0[6] | 5.0[6] | 6.4 | - | 6.1 | - | 7 |
| Phosphorus | mg/L | 0.1 | na | na | nd | nd | nd | nd | nd |
| Phosphorus, Total | mg/L | 0.01 | na | na | 0.05 | - | 0.04 | - | 0.02 |
| Potassium | mg/L | 0.5 | na | na | 1.2 | nd | nd | nd | 0.7 |
| Reactive Silica($SiO_2$) | mg/L | 0.5 | na | na | 4.6 | - | 4.9 | - | 7.2 |
| Selenium | mg/L | 0.002 | na | na | nd | nd | nd | nd | nd |
| Silver | mg/L | 0.0005/0.00005 | na | na | <0.0005 | <0.0005 | <0.00005 | <0.00005 | <0.00005 |
| Sodium | mg/L | 0.1 | na | na | 1.9 | 1.9 | 2 | 2.1 | 2.2 |
| Strontium | mg/L | 0.005 | na | na | 0.011 | 0.011 | 0.013 | 0.013 | 0.019 |
| Sulphate | mg/L | 2 | na | na | 8 | - | 11 | - | 16 |
| Thallium | mg/L | 0.0001 | na | na | nd | nd | nd | nd | 0.0001 |
| Tin | mg/L | 0.002 | na | na | nd | nd | nd | nd | nd |
| Titanium | mg/L | 0.002 | na | na | nd | nd | 0.003 | nd | 0.003 |
| Total Dissolved Solids(Calculated) | mg/L | 1 | na | na | - | 24 | - | 31 | - |
| Total Kjeldahl Nitrogen(as N) | mg/L | 0.05 | na | na | 0.58 | - | 0.39 | - | 0.43 |
| Total Suspended Solids | mg/L | 5/1 | 25.0 | 50.0 | <5 | - | 3 | - | 2 |
| Turbidity | NTU | 0.1 | na | na | 1.1 | - | 3 | - | 1.9 |
| Uranium | mg/L | 0.0001 | na | na | nd | nd | nd | nd | 0.0001 |
| Vanadium | mg/L | 0.002 | na | na | nd | nd | nd | nd | nd |
| Zinc | mg/L | 0.002/0.001 | 0.5 | 1.0 | 0.168 | 0.171 | 0.36 | 0.363 | 0.44 |

[1] LOQ = Limit of Quantitation = lowest level of the parameter that can be quantified with confidence.

[2] MMLER = *Metal Mining Liquid Effluent Regulations* (Fisheries Act, 1994)

[3] na = Regulation values not available

[4] - = Not Analyzed

[5] nd = Parameter not detected

[6] pH limits listed are minimum.

**Table 4.4: Total versus Dissolved Concentrations for Selected Metals in Samples Collected at Heath Steele, 20 August 1997.**

| | | | REFERENCE STATIONS | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Parameters | Units | LOQ[1] | HR1A Total | HR1A Dissolved | HR1B Total | HR1B Dissolved | HR2A Total | HR2A Dissolved | HR2B Total | HR2B Dissolved | HR3A Total | HR3A Dissolved | HR3B Total | HR3B Dissolved |
| Aluminum | mg/L | 0.005 | 0.031 | 0.019 | 0.047 | 0.018 | 0.049 | 0.021 | 0.046 | 0.021 | 0.033 | 0.013 | 0.059 | 0.013 |
| Cadmium | mg/L | 0.00005 | nd 2 | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd |
| Copper | mg/L | 0.0003 | nd | nd | nd | nd | 0.0003 | 0.0003 | 0.0004 | 0.0004 | nd | nd | nd | nd |
| Iron | mg/L | 0.02 | 0.09 | 0.05 | 0.08 | 0.05 | 0.1 | 0.07 | 0.13 | 0.07 | 0.14 | 0.09 | 0.18 | 0.09 |
| Lead | mg/L | 0.0001 | nd | nd | nd | nd | nd | nd | 0.0003 | nd | nd | nd | nd | nd |
| Zinc | mg/L | 0.001 | 0.008 | 0.003 | 0.009 | 0.003 | 0.016 | 0.012 | 0.017 | 0.018 | 0.003 | nd | 0.004 | nd |

| | | | EXPOSURE STATIONS | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Parameters | Units | LOQ[1] | HE1A Total | HE1A Dissolved | HE1B Total | HE1B Dissolved | HE2A Total | HE2A Dissolved | HE2B Total | HE2B Dissolved | HE3A Total | HE3A Dissolved | HE3B Total | HE3B Dissolved | HE4A Total | HE4A Dissolved | HE4B Total | HE4B Dissolved | HE5A Total | HE5A Dissolved | HE5B Total |
| Aluminum | mg/L | 0.005 | 0.322 | 0.185 | 0.277 | 0.173 | 0.247 | 0.153 | 0.169 | 0.118 | 0.15 | 0.065 | 0.082 | 0.06 | 0.074 | 0.058 | 0.07 | 0.056 | 0.059 | 0.046 | 0.055 |
| Cadmium | mg/L | 0.00005 | 0.00032 | 0.00032 | 0.00022 | 0.00022 | 0.00021 | 0.00022 | 0.0002 | 0.0002 | 0.00016 | 0.00011 | 0.00011 | 0.00012 | 0.0001 | 0.00011 | 0.0001 | 0.00011 | 0.00008 | 0.00009 | 0.00007 |
| Copper | mg/L | 0.0003 | 0.0225 | 0.0201 | 0.0193 | 0.0167 | 0.018 | 0.0151 | 0.0158 | 0.0141 | 0.0098 | 0.0071 | 0.0075 | 0.007 | 0.0073 | 0.007 | 0.0071 | 0.0068 | 0.0062 | 0.0059 | 0.0057 |
| Iron | mg/L | 0.02 | 0.67 | 0.32 | 0.54 | 0.29 | 0.5 | 0.26 | 0.42 | 0.24 | 0.36 | 0.15 | 0.24 | 0.16 | 0.23 | 0.16 | 0.22 | 0.16 | 0.18 | 0.14 | 0.17 |
| Lead | mg/L | 0.0001 | 0.003 | 0.001 | 0.0025 | 0.001 | 0.0027 | 0.0008 | 0.0019 | 0.0008 | 0.0022 | 0.0004 | 0.0009 | 0.0004 | 0.0008 | 0.0004 | 0.0007 | 0.0004 | 0.0005 | 0.0002 | 0.0004 |
| Zinc | mg/L | 0.001 | 0.157 | 0.158 | 0.111 | 0.113 | 0.106 | 0.109 | 0.107 | 0.111 | 0.085 | 0.074 | 0.066 | 0.066 | 0.062 | 0.064 | 0.068 | 0.071 | 0.058 | 0.061 | 0.061 |

[1] LOQ = Limit of Quantitation = lowest level of the parameter that can be quantified with confidence

[2] nd = Parameter not detected

**Table 4.4: Total versus Disso**

| Parameters | Units | LOQ[1] |
|---|---|---|
| Aluminum | mg/L | 0.005 |
| Cadmium | mg/L | 0.00005 |
| Copper | mg/L | 0.0003 |
| Iron | mg/L | 0.02 |
| Lead | mg/L | 0.0001 |
| Zinc | mg/L | 0.001 |

| Parameters | Units | LOQ[1] | HE5B Dissolved |
|---|---|---|---|
| Aluminum | mg/L | 0.005 | 0.042 |
| Cadmium | mg/L | 0.00005 | 0.00008 |
| Copper | mg/L | 0.0003 | 0.0057 |
| Iron | mg/L | 0.02 | 0.13 |
| Lead | mg/L | 0.0001 | 0.0002 |
| Zinc | mg/L | 0.001 | 0.062 |

[1] LOQ = Limit of Quantitation = l

[2] nd = Parameter not detected

| HSE-3 (Dissolved) 97/11/13 |
| --- |
| 10 |
| - |
| 0.36 |
| - |
| <0.0005 |
| nd |
| 0.007 |
| nd |
| - |
| nd |
| nd |
| 0.00078 |
| 4.3 |
| - |
| - |
| <0.0005 |
| 0.0063 |
| - |
| - |
| 0.041 |
| 0.3 |
| 5.4 |
| - |
| 0.14 |
| 0.0015 |
| 1.2 |
| 0.17 |
| nd |
| 0.0002 |
| 0.002 |
| 0.42 |
| nd |
| nd |
| - |
| nd |
| - |
| 0.7 |
| - |
| nd |
| <0.00005 |
| 2.3 |
| 0.015 |
| - |
| nd |
| nd |
| 0.002 |
| 38 |
| - |
| - |
| - |
| nd |
| nd |
| 0.37 |

the mine, with the highest concentrations in reach HE1 and the lowest downstream concentrations in HE5. All of these parameters except aluminum remained elevated relative to reference site values in the final exposure reach (HE5), and all occurred in excess of Canadian surface water quality guidelines (CCREM, 1987) in some or all downstream reaches. Dissolved and total metal concentrations were similar for cadmium, copper and zinc, whereas dissolved metal concentrations were substantially lower than total metal concentrations for lead, iron and aluminum. On some occasions, dissolved metal concentrations were slightly higher than totals due to either the precision of the analytical method or because the values were close to the detection limit.

In terms of general water quality conditions, water hardness was low throughout (≤20 mg/L as $CaCO_3$) in Tomogonops River reaches, but was somewhat higher (about 30 mg/L $CaCO_3$) in reference reach HR3 in the neighbouring Little River (Table 4.3). Conductivity and sulphate levels were relatively low, but showed some elevation in near-field reaches (HE1 and HE2). Field pH levels were near neutral (pH ~6.7 to 7.1) throughout.

Based on these results, it may be concluded that the field program was successful in sampling an aqueous metal gradient downstream of Heath Steele, with concentrations of some metals (e.g., Zn, Cu, Pb) often at least an order of magnitude higher in the near-field (HE1) than at reference sites.

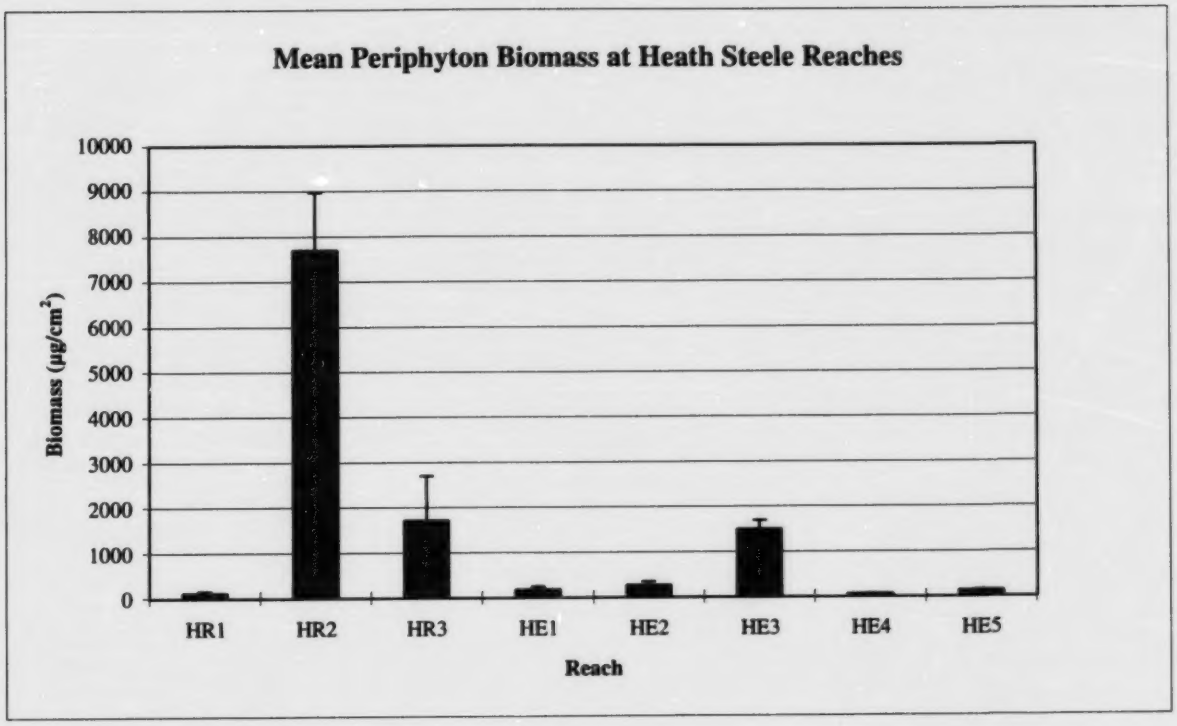
## 4.3 Periphyton

Detailed biological analyses of periphyton, as provided by Dr. H.C. Duthie, are provided in Annex 1. A summary of results in terms of numbers of taxa and biomass by reach is presented in Figure 4.3. Table 4.5 and Figure 4.4 present periphyton metal concentration data.

Periphyton samples were rich in algal species and variable in terms of biomass. Spatial trends among the exposure and reference reaches are not readily apparent in the data (Figure 4.3).

Periphyton copper, cadmium, lead and zinc concentrations all showed a reference-exposure difference, with an exposure area gradient also indicated for lead (Figure 4.4 vs Figure 4.2) and also iron (Table 4.5). Concentration ranges for important metals, including cadmium, copper and zinc, were often variable between samples within reaches.

## 4.4 Benthic Invertebrates


Mean Periphyton Biomass at Heath Steele Reaches
Biomass (µg/cm²)
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
HR1
HR2
HR3
HE1
HE2
HE3
HE4
HE5
Reach

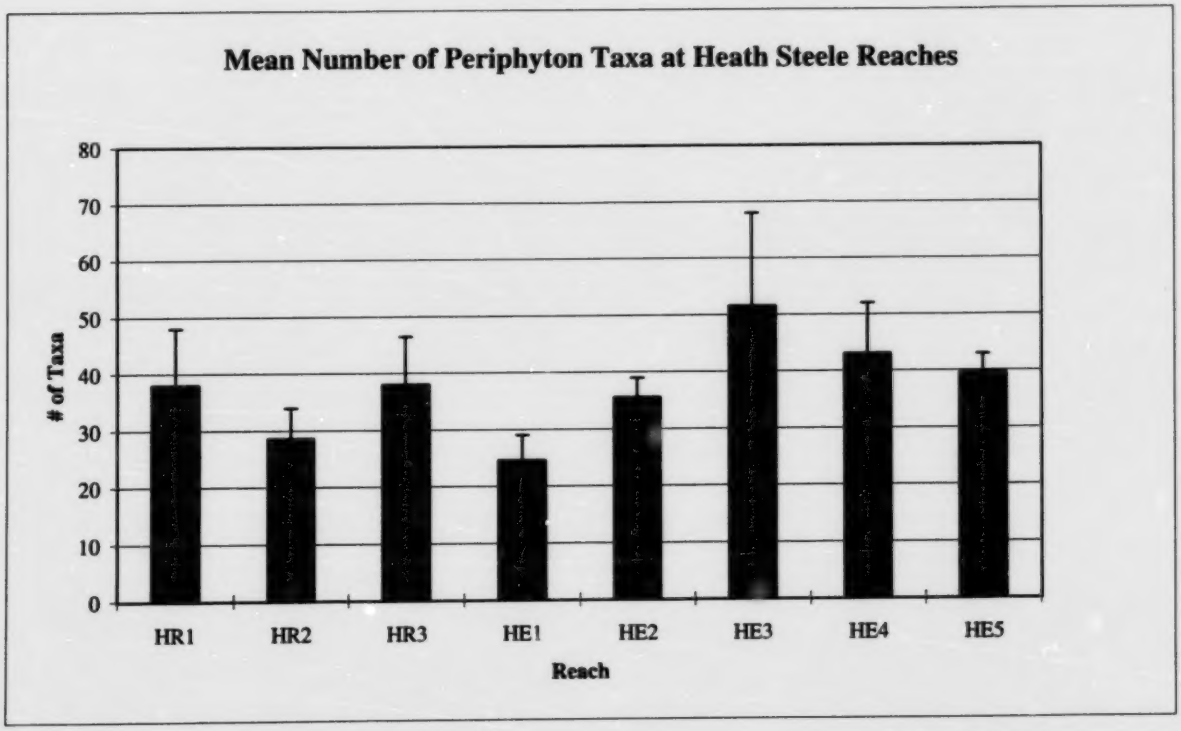

Mean Number of Periphyton Taxa at Heath Steele Reaches
# of Taxa
80
70
60
50
40
30
20
10
0
HR1
HR2
HR3
HE1
HE2
HE3
HE4
HE5
Reach

**Table 4.5: Concentrations of Selected Metals in Periphyton collected at Heath Steele, August 1997.**

All values expressed on a dry mass basis.

| | | | REFERENCE STATIONS | | | | | | EXPOSURE STATIONS | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | HR1A | HR1B | HR2A | HR2B | HR3A | HR3B | HE1A | HE1B | HE2A | HE2B | HE3A | HE3B | HE4A | HE4B | HE5A | HE5B |
| Parameters | Units | MDL[1] | 97/08/18 | 97/08/18 | 97/08/15 | 97/08/15 | 97/08/17 | 97/08/19 | 97/08/13 | 97/08/16 | 97/08/16 | 97/08/14 | 97/08/14 | 97/08/14 | 97/08/16 | 97/08/13 | 97/08/17 | 97/08/19 |
| Aluminum | mg/kg | 0.5 | 450 | 4700 | 520 | 2600 | 7800 | 11000 | 22000 | 17000 | 18000 | 12000 | 500 | 16000 | 1800 | 15000 | 940 | 18000 |
| Antimony | mg/kg | 0.01 | <0.10 | 0.16 | <0.10 | 0.17 | <0.10 | <0.10 | 1.3 | 0.96 | 0.9 | 0.67 | <0.10 | 0.51 | 0.19 | 0.13 | <0.10 | 0.31 |
| Arsenic | mg/kg | 0.1 | 0.43 | 7.3 | 1 | 14 | 9 | 22 | 120 | 990 | 100 | 60 | 2.9 | 64 | 9.5 | 20 | 3.1 | 100 |
| Barium | mg/kg | 0.05 | 13 | 150 | 55 | 1500 | 32 | 260 | 140 | 120 | 140 | 140 | 7.1 | 330 | 220 | 110 | 11 | 980 |
| Beryllium | mg/kg | 0.01 | <0.10 | 0.41 | <0.10 | 0.23 | 0.37 | 0.43 | 1.8 | 1.7 | 1.6 | 1.5 | <0.10 | 1.3 | 0.17 | 0.59 | <0.10 | 1.7 |
| Bismuth | mg/kg | 0.01 | <0.10 | 0.26 | <0.10 | 0.16 | 0.11 | 0.21 | 13 | 9 | 8.4 | 4.5 | 0.19 | 3.6 | 0.53 | 0.42 | 0.16 | 1 |
| Boron | mg/kg | 0.2 | <2.0 | 1.1 | <2.0 | <2.0 | <2.0 | <2.0 | 4.7 | 7.6 | 2.5 | 4.4 | <2.0 | 2.7 | <2.0 | 1.7 | <2.0 | 3.4 |
| Cadmium | mg/kg | 0.005 | 0.31 | 3.5 | 0.26 | 3.9 | 0.21 | 1.7 | 9.2 | 11 | 14 | 22 | 0.75 | 28 | 2.2 | 9.2 | 0.74 | 70 |
| Chromium | mg/kg | 0.05 | 0.52 | 2.9 | 1.3 | 4.6 | 14 | 23 | 18 | 13 | 21 | 11 | 0.66 | 14 | 11 | 71 | 2 | 22 |
| Cobalt | mg/kg | 0.01 | 1.2 | 13 | 2.4 | 23 | 6.1 | 15 | 110 | 130 | 170 | 390 | 11 | 570 | 49 | 160 | 9.8 | 1200 |
| Copper | mg/kg | 0.03 | 1.2 | 15 | 1.3 | 15 | 6.2 | 11 | 930 | 1000 | 980 | 1100 | 32 | 830 | 77 | 250 | 24 | 1600 |
| Iron | mg/kg | 2 | 1000 | 10000 | 1100 | 6000 | 17000 | 21000 | 46000 | 42000 | 39000 | 27000 | 1800 | 30000 | 4300 | 24000 | 1700 | 25000 |
| Lead | mg/kg | 0.01 | 4.2 | 52 | 1.8 | 23 | 8.6 | 16 | 1100 | 770 | 760 | 500 | 18 | 430 | 58 | 120 | 17 | 380 |
| Manganese | mg/kg | 0.05 | 700 | 7600 | 350 | 5100 | 410 | 4400 | 3300 | 4200 | 6100 | 12000 | 500 | 26000 | 2000 | 6700 | 610 | 60000 |
| Molybdenum | mg/kg | 0.01 | 0.11 | 0.81 | <0.10 | 0.69 | 0.42 | 0.74 | 1.6 | 1.7 | 1.5 | 1.3 | <0.10 | 1.6 | 0.66 | 1.2 | <0.10 | 3.1 |
| Nickel | mg/kg | 0.05 | 0.49 | 4 | 0.99 | 5.2 | 11 | 19 | 19 | 21 | 26 | 38 | 1.8 | 33 | 3.3 | 50 | 1.7 | 73 |
| Selenium | mg/kg | 0.2 | <2.0 | <2.0 | <2.0 | 1.3 | <2.0 | <2.0 | 3.5 | 3.5 | 3.3 | 3.7 | <2.0 | 3.7 | <2.0 | 1.3 | < | 3.3 |
| Silver | mg/kg | 0.005 | <0.050 | 0.32 | <0.050 | 0.11 | <0.050 | 0.1 | 3.6 | 2.6 | 2.6 | 1.5 | 0.088 | 1.3 | 0.15 | 0.21 | 0.053 | 0.24 |
| Strontium | mg/kg | 0.05 | 2.9 | 33 | 1.4 | 32 | 5.9 | 17 | 24 | 27 | 30 | 38 | 1.7 | 42 | 7.1 | 21 | 2.1 | 55 |
| Thallium | mg/kg | 0.01 | <0.10 | 0.44 | <0.10 | 0.25 | <0.10 | 0.14 | 0.82 | 0.59 | 0.61 | 0.32 | <0.10 | 0.61 | <0.10 | 0.21 | <0.10 | 0.8 |
| Tin | mg/kg | 0.01 | 0.2 | 0.17 | <0.10 | 1.3 | 0.21 | 0.14 | 0.86 | 0.79 | 0.61 | 0.47 | 0.13 | 0.32 | 1.3 | 0.36 | <0.10 | 0.79 |
| Titanium | mg/kg | 0.03 | 78 | 360 | 71 | 250 | 300 | 370 | 560 | 520 | 660 | 360 | 16 | 470 | 72 | 280 | 61 | 430 |
| Vanadium | mg/kg | 0.05 | 2.4 | 23 | 2 | 9.3 | 19 | 32 | 37 | 34 | 38 | 23 | 1.2 | 29 | 3.8 | 52 | 2.4 | 32 |
| Zinc | mg/kg | 0.1 | 26 | 620 | 65 | 1200 | 76 | 220 | 3400 | 4300 | 5300 | 9100 | 300 | 8800 | 1000 | 2400 | 310 | 13000 |

[1] MDL - Method Detection Limit - lowest level the parameter can be detected with confidence

Benthic community sample composition is presented in detail in Appendix 4, with Table 4.6 providing a summary by reach and expressed per square metre. Figure 4.5 illustrates spatial trends in benthic community indices by reach.

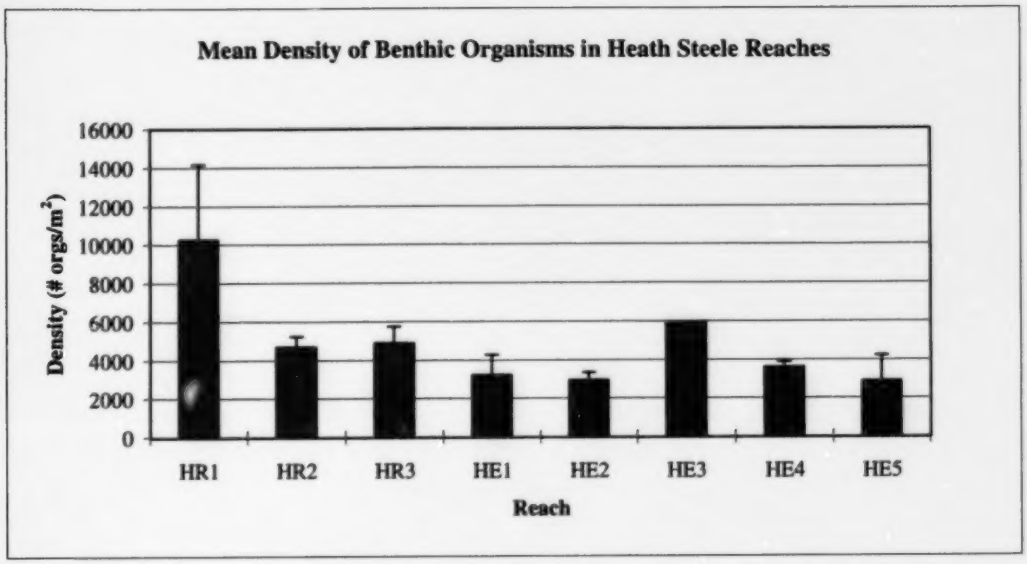
Overall, riffle communities in all reaches were rich in species and numbers of benthic organisms. Chironomids were generally predominant, although EPT taxa (Ephemeroptera-Plecoptera-Trichoptera) were well represented. These latter groups are generally considered to be sensitive to metals. Mean total densities of organisms were relatively high in all reaches, at about 3,000 to 10,000 organisms per square metre (Figure 4.5).

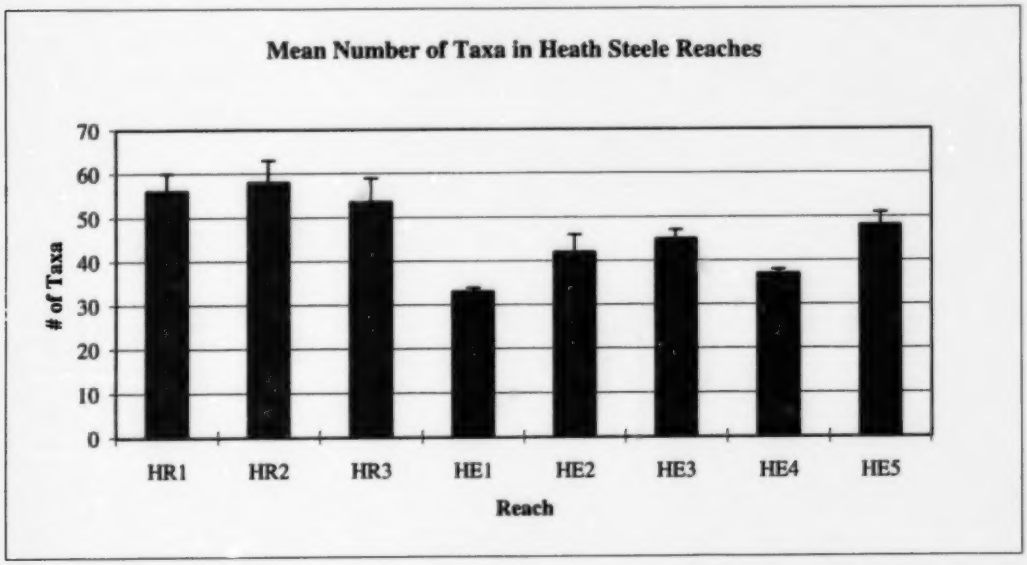
Spatial trends are apparent in terms of the EPT Index (number of EPT taxa) and total number of taxa present, with a suppression in values at reach HE1 in the near-field, and recovery to reference site conditions in the downstream reach, HE5. Other trends are apparent for individual taxa, such as the chironomids *Micropsectra* which was common everywhere except in the near-field reaches, and *Rheocricotopus* which showed the opposite trend (i.e., densities were highest in the near-field and were lower in the far-field). Percent Orthocladiinae reflected the trend seen for *Rheocricotopus*. Except for a very high total organism density at HR1, no spatial trends are apparent in total density.

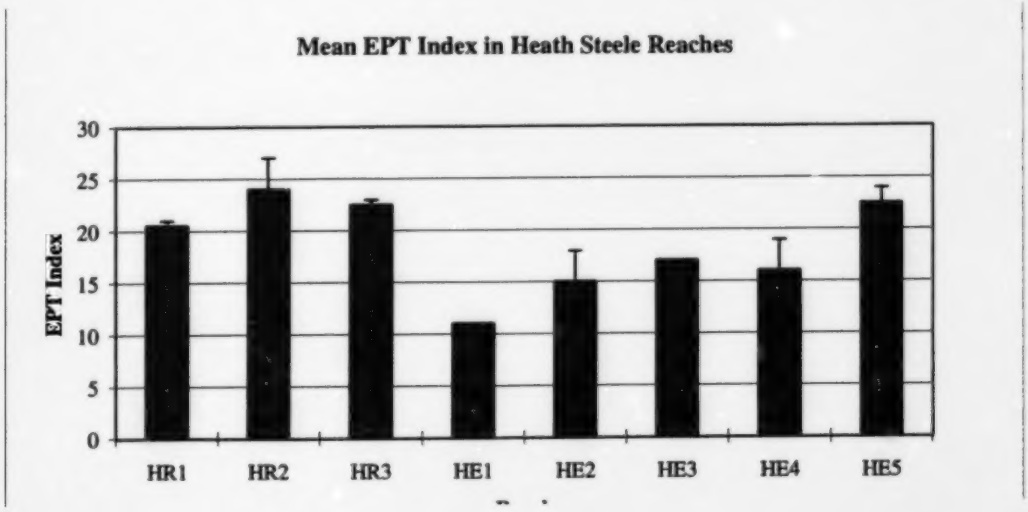
As illustrated in Appendix 4, the incidence of abnormalities in chironomid head capsules was relatively low throughout, with no obvious spatial trend across the water quality gradient downstream of Heath Steele.

**Table 4.6: Benthic Community Indices, Based on T-Sampler Collections, Heath Steele, August 1997.**

| Station | Total Density (no./$m^2$) | Number of Taxa | EPT Index | Orthocladiinae (%) | Micropsectra (%) | Rheocricotopus (%) |
|---|---|---|---|---|---|---|
| HR1A | 6360 | 52 | 20 | 5 | 6.2 | 0.1 |
| HR1B | 14159 | 60 | 21 | 23 | 12.1 | 0.1 |
| HR2A | 5232 | 53 | 21 | 15 | 6.7 | 0.6 |
| HR2B | 4091 | 63 | 27 | 11 | 8.0 | 0.4 |
| HR3A | 3976 | 48 | 23 | 10 | 26.8 | 0.0 |
| HR3B | 5764 | 59 | 22 | 16 | 9.3 | 0.7 |
| HE1A | 4272 | 34 | 11 | 74 | 0.2 | 11.4 |
| HE1B | 2148 | 32 | 11 | 66 | 0.4 | 4.7 |
| HE2A | 2534 | 38 | 12 | 44 | 0.2 | 4.6 |
| HE2B | 3359 | 46 | 18 | 27 | 1.0 | 5.9 |
| HE3A | 5912 | 47 | 17 | 12 | 10.0 | 0.9 |
| HE3B | 5976 | 43 | 17 | 6 | 17.4 | 1.3 |
| HE4A | 3277 | 38 | 19 | 4 | 12.2 | 0.0 |
| HE4B | 3896 | 36 | 13 | 11 | 14.4 | 1.4 |
| HE5A | 1497 | 51 | 24 | 7 | 10.8 | 0.4 |
| HE5B | 4229 | 45 | 21 | 18 | 10.6 | 0.9 |


Mean Density of Benthic Organisms in Heath Steele Reaches
Density (# orgs/m²)
16000
14000
12000
10000
8000
6000
4000
2000
0
HR1
HR2
HR3
HE1
HE2
HE3
HE4
HE5
Reach


Mean Number of Taxa in Heath Steele Reaches
# of Taxa
70
60
50
40
30
20
10
0
HR1
HR2
HR3
HE1
HE2
HE3
HE4
HE5
Reach


Mean EPT Index in Heath Steele Reaches
EPT Index
30
25
20
15
10
5
0
HR1
HR2
HR3
HE1
HE2
HE3
HE4
HE5

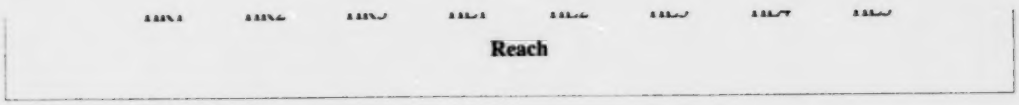



**Figure 4.5: Mean Values of Selected Benthic Indices, Heath Steele, August 1997.**
Reach Means (±1 S.E.)

## 4.5 Fish

### 4.5.1 Fish Catches

Detailed electrofishing results in terms of species, size, numbers and ages of fish are presented in Appendix 5. Table 4.7 summarizes the numbers of fish captured at each station, while Tables 4.8 and 4.9 provide CPUE (numbers of fish per minute) and BPUE (biomass of fish per minute), respectively. Summaries of the data in Tables 4.7 to 4.9 are illustrated graphically in Figures 4.6 and 4.7.

Ten species of fish were represented in the fish collections, with juvenile Atlantic salmon, blacknose dace and brook trout generally the most abundant. Fish CPUE and BPUE were lowest at HE1, but recovered in the downstream direction, and appeared to track the metal concentration gradient in the water. No fish were found at HE1A in the upstream extremity of reach HE1, although caged Atlantic salmon survived here over nine days.

Juvenile Atlantic salmon were most abundant at HR3 (Little River) and HE5, which are unaffected by migration barriers. Salmon densities were much lower at HE3 and HE4 than at HE5, mainly due to an absence of any salmon fry (age 0+) upstream of the abandoned railway crossing. No Atlantic salmon were captured in the Little South Branch Tomogonops (HE1, HE2) possibly due to an avoidance reaction, although other species were found in low numbers in these reaches. As expected, salmon were also absent at reference reach HR1.

Blacknose dace were most abundant at HR2 and HE5 but, unlike salmon, were found in all reaches.

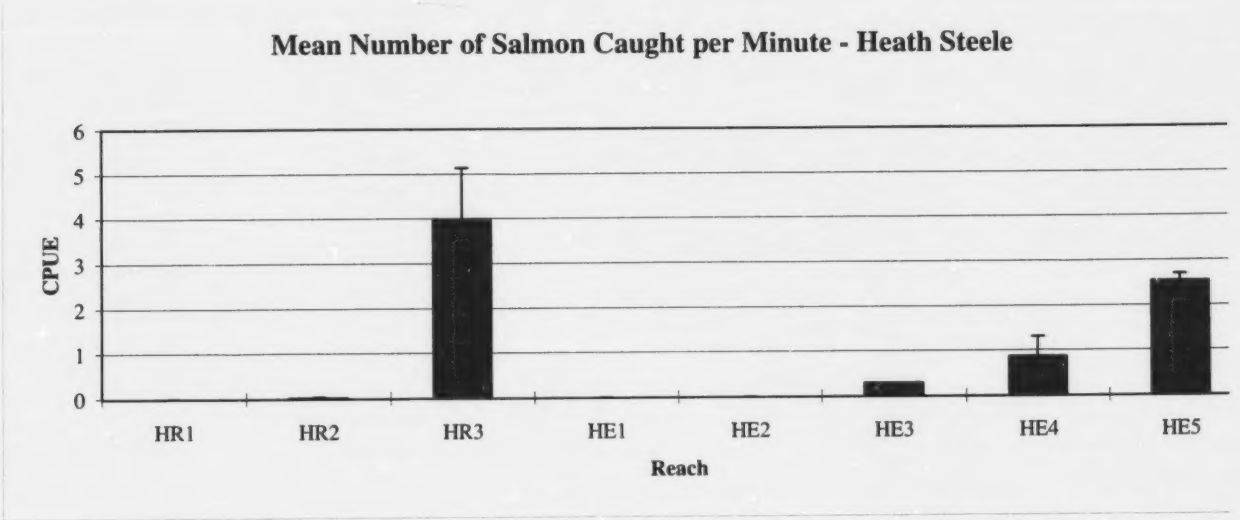
### 4.5.2 Atlantic Salmon and Blacknose Dace Growth

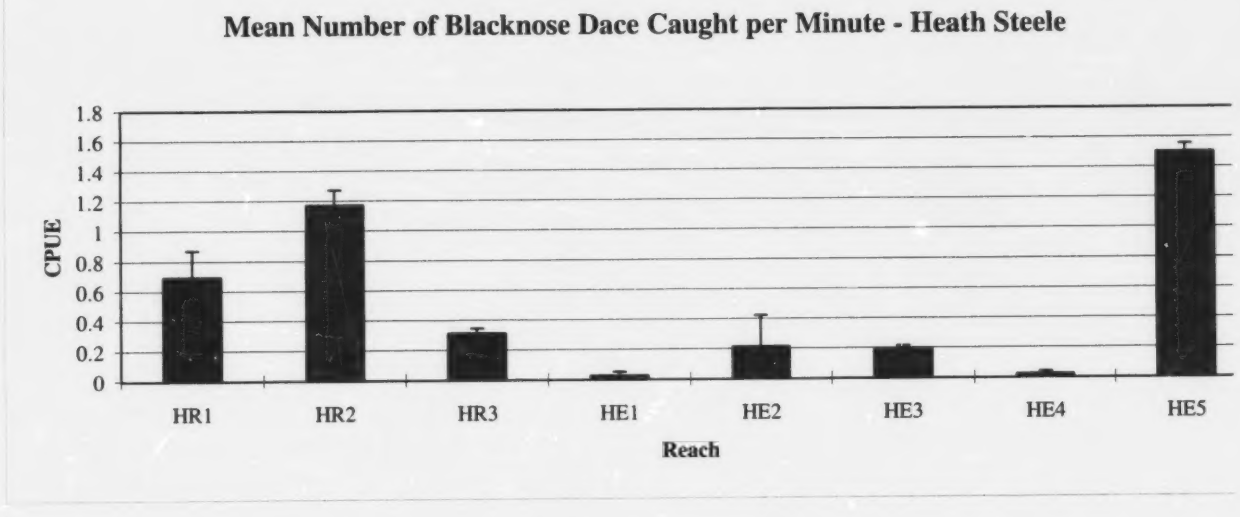
Data on ages for selected specimens of fish are presented in Appendix 5, which include length-frequency histograms and raw data for all fish specimens. The size-frequency plots for all dace and all salmon show definite break points separating fry (0+ fish) from older age classes. Older age classes of salmon and dace are less distinct. As shown in Appendix 5, juvenile salmon were present in four age classes, although age 3+ salmon (the oldest age class) were low in abundance. Blacknose dace spanned six year-classes, with overlaps in length between age classes for fish aged 1+ and older.

**Table 4.8: Catch per unit effort (CPUE) of fish at Heath Steele, August 1997.**

Values are number of fish per minute of electrofishing.

| Area | Station | Sampling Date | Electrofishing Effort (seconds) | Atlantic Salmon (fish/min) | Blacknose Dace (fish/min) | Brook Trout (fish/min) | Lake Chub (fish/min) | Slimy Scuplin (fish/min) | White Sucker (fish/min) | Creek Chub (fish/min) | 3-Spine Stickleback (fish/min) | 9-Spine Stickleback (fish/min) | Sea Lamprey (fish/min) | All Fish (fish/min) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Reference** | HR1A | 18-Aug-97 | 1402 | 0.000 | 0.514 | 6.163 | 2.183 | 0.000 | 0.342 | 0.000 | 0.000 | 0.000 | 0.000 | 9.201 |
| | HR1B | 18-Aug-97 | 1723 | 0.000 | 0.871 | 4.597 | 1.010 | 0.000 | 0.070 | 0.070 | 0.000 | 0.000 | 0.000 | 6.616 |
| | | **Mean** | **1562.5** | **0.000** | **0.692** | **5.380** | **1.596** | **0.000** | **0.206** | **0.035** | **0.000** | **0.000** | **0.000** | **7.909** |
| | HR2A | 15-Aug-97 | 1699 | 0.000 | 1.271 | 0.706 | 0.000 | 1.307 | 0.000 | 0.177 | 0.000 | 0.000 | 0.000 | 3.461 |
| | HR2B | 15-Aug-97 | 1750 | 0.034 | 1.063 | 1.749 | 0.000 | 0.686 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 3.531 |
| | | **Mean** | **1724.5** | **0.017** | **1.167** | **1.227** | **0.000** | **0.996** | **0.000** | **0.088** | **0.000** | **0.000** | **0.000** | **3.496** |
| | HR3A | 17-Aug-97 | 1721 | 2.824 | 0.279 | 0.139 | 0.000 | 0.558 | 0.279 | 0.000 | 0.244 | 0.000 | 0.174 | 4.497 |
| | HR3B | 19-Aug-97 | 1565 | 5.137 | 0.345 | 0.230 | 0.000 | 0.613 | 0.000 | 0.000 | 0.000 | 0.000 | 0.115 | 6.441 |
| | | **Mean** | **1643.0** | **3.981** | **0.312** | **0.185** | **0.000** | **0.586** | **0.139** | **0.000** | **0.122** | **0.000** | **0.145** | **5.469** |
| | All | **Mean** | **1643.3** | **1.333** | **0.724** | **2.264** | **0.532** | **0.527** | **0.115** | **0.041** | **0.041** | **0.000** | **0.048** | **5.625** |
| **Exposure** | HE1A | 13-Aug-97 | 999 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| | HE1B | 16-Aug-97 | 1231 | 0.000 | 0.049 | 0.049 | 0.049 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.146 |
| | | **Mean** | **1115.0** | **0.000** | **0.024** | **0.024** | **0.024** | **0.000** | **0.000** | **0.000** | **0.000** | **0.000** | **0.000** | **0.073** |
| | HE2A | 16-Aug-97 | 1555 | 0.000 | 0.000 | 0.154 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.154 |
| | HE2B | 14-Aug-97 | 1565 | 0.000 | 0.422 | 0.192 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.613 |
| | | **Mean** | **1560.0** | **0.000** | **0.211** | **0.173** | **0.000** | **0.000** | **0.000** | **0.000** | **0.000** | **0.000** | **0.000** | **0.384** |
| | HE3A | 14-Aug-97 | 1775 | 0.270 | 0.169 | 1.318 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 1.758 |
| | HE3B | 14-Aug-97 | 1676 | 0.286 | 0.215 | 0.788 | 0.000 | 0.000 | 0.036 | 0.036 | 0.000 | 0.000 | 0.000 | 1.360 |
| | | **Mean** | **1725.5** | **0.278** | **0.192** | **1.053** | **0.000** | **0.000** | **0.018** | **0.018** | **0.000** | **0.000** | **0.000** | **1.559** |
| | HE4A | 16-Aug-97 | 1667 | 0.396 | 0.000 | 0.540 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.936 |
| | HE4B | 13-Aug-97 | 1542 | 1.323 | 0.039 | 0.428 | 0.039 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 1.829 |
| | | **Mean** | **1604.5** | **0.859** | **0.019** | **0.484** | **0.019** | **0.000** | **0.000** | **0.000** | **0.000** | **0.000** | **0.000** | **1.382** |
| | HE5A | 17-Aug-97 | 1816 | 2.709 | 1.553 | 0.463 | 0.396 | 0.165 | 0.000 | 0.099 | 0.000 | 0.033 | 0.000 | 5.419 |
| | HE5B | 19-Aug-97 | 1924 | 2.370 | 1.435 | 0.187 | 0.249 | 0.000 | 0.031 | 0.031 | 0.000 | 0.000 | 0.000 | 4.304 |
| | | **Mean** | **1870.0** | **2.540** | **1.494** | **0.325** | **0.323** | **0.083** | **0.016** | **0.065** | **0.000** | **0.017** | **0.000** | **4.861** |
| | All | **Mean** | **1575.000** | **0.736** | **0.388** | **0.412** | **0.073** | **0.017** | **0.007** | **0.017** | **0.000** | **0.003** | **0.000** | **1.652** |



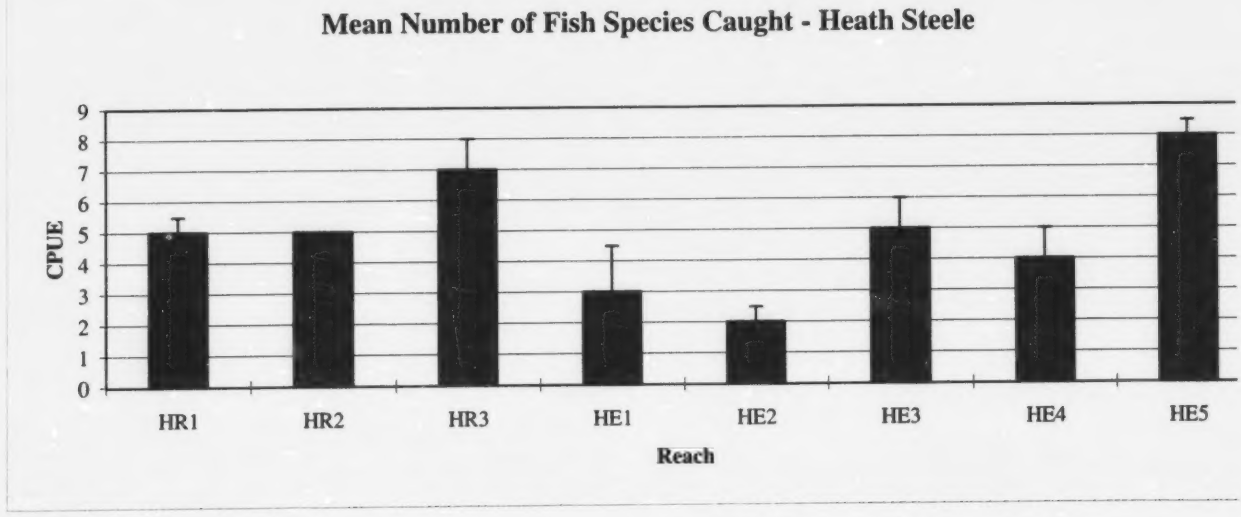
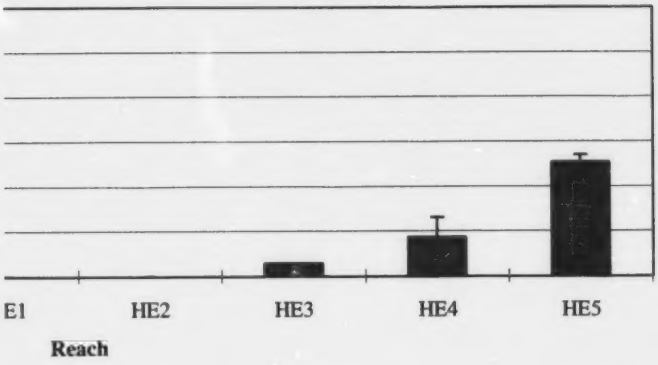
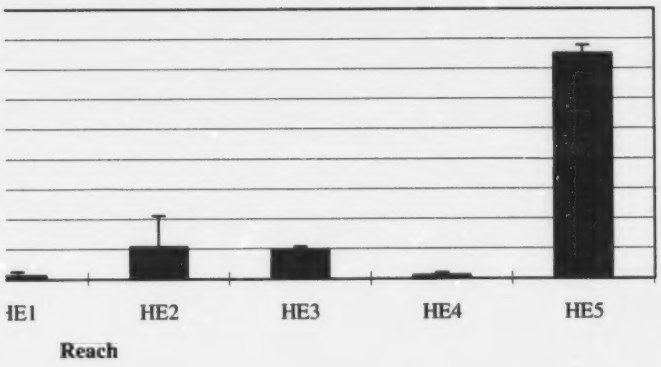




**Figure 4.7: Mean Numbers of Atlantic Salmon, Blacknose Dace and Mean Numbers of Fish Species Captured by Electrofishing, Heath Steele, August 1997.**
Reach Means (±1 S.E.) Based on Data in Tables 4.8 and 4.9.

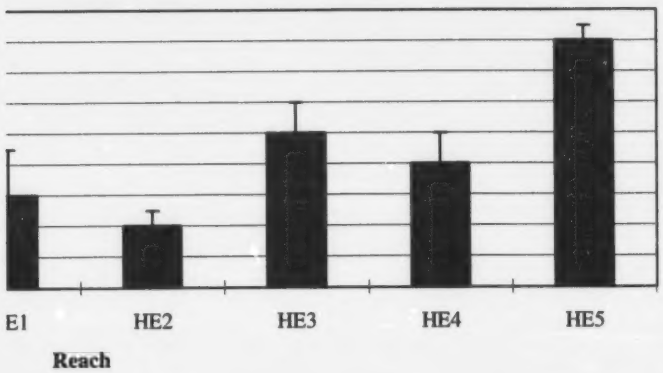
## Caught per Minute - Heath Steele




## ace Caught per Minute - Heath Steele




## Species Caught - Heath Steele



The only spatial difference readily apparent in fish size at age is seen in comparison of salmon fry at HR3 (Little River) and HE5 (see Table 4.10a for a summary of biological characteristics of fish captured at Heath Steele). Fry at HR3 were smaller than those at HE5 (compare reach-specific length-frequency plots in Appendix 5). This effect could be attributed to the higher densities of fry at HR3 and a density-dependent effect on growth (e.g., competition for food).

### 4.5.3 Caged Atlantic Salmon

Biological measurements taken on caged Atlantic salmon used in tissue analysis are presented in Appendix 5. As noted in Section 2.0, all fish were yearlings from Heath Steele's McCormack Reservoir rearing facility. These yearlings were substantially larger than wild yearlings from the Tomogonops River (refer to Appendix 5 for fish sizes).

All fish survived the nine-day exposure at all reference and exposure sites, including HE1A where wild fish were apparently absent.

### 4.5.4 Metals and Metallothionein

Results of metallothionein (MT) and metal analyses on wild juvenile Atlantic salmon, caged juvenile Atlantic salmon and blacknose dace are summarized in Tables 4.10 to 4.12 and in Figures 4.8 to 4.12, with data on metals provided for zinc, copper, cadmium and lead. Table 4.13 and Figures 4.8 to 4.12 also present tissue data for juvenile brook trout, which were sampled opportunistically as a potential alternate sentinel species. Data on MT and metals in caged Atlantic salmon gill tissue were also provided by the Department of Fisheries and Oceans, based on pooling of the gill tissues from both caged fish for each station.

Table 4.14 presents a detailed tabulation of all fish tissue results including all metals analyzed, as well as a correlation matrix for MT and metals in tissues. The correlation matrix does not form part of formal hypothesis testing, but is useful in identifying possible cause-effect linkages between tissue metal concentrations and MT concentrations. In general, the best correlations are with cadmium, and metals were most often correlated with MT in gill in caged salmon.

Examination of the data shows that tissue MT levels were higher in all species at Heath Steele exposure stations than at reference sites, at least in the near-field. MT results for viscera and gill in caged juvenile salmon closely match the aqueous metal gradient measured downstream of the mine (Figure 4.8, Section 4.5.4). Visceral MT levels in wild fish were generally highest in

blacknose dace and lowest in juvenile Atlantic salmon, with an intermediate level present in brook trout.

The effect of fish size or age on MT or tissue metal concentration was not specifically tested. However, inspection of the data (Tables 4.10 to 4.12) and fish measurements presented in Appendix 6 does not suggest any effect of fish size on tissue response in these samples. The blacknose dace data are less conclusive in this regard, as composite samples analyzed often included a mixture of fish sizes and ages, reflecting the availability of fish in each sample.

Caged Atlantic salmon had lower visceral MT levels than did wild salmon, and gill MT levels were less than those found in viscera.

In terms of visceral metal concentrations, exposure area-reference area differences are apparent in wild fish, with higher concentrations occurring in exposed fish for some metals. These differences are evaluated with respect to statistical significance in the hypothesis testing section (Section 5.2.1). Wild Atlantic salmon viscera had higher metal concentrations than did blacknose dace viscera for zinc and copper. In caged salmon, visceral copper, cadmium and lead levels were all low relative to levels in wild fish of either species, while visceral zinc concentrations in caged fish were intermediate between those seen in blacknose dace and wild salmon.

# 5.0 HYPOTHESIS TESTING

## 5.1 Methods

The eight hypotheses considered testable at Heath Steele are listed in Table 5.1, along with a more specific listing of the "effect" (response) and "exposure" (predictor) variables to be examined under each hypothesis. The general reasoning behind all of these hypotheses is that a mine "effect" is a measurable difference between reference and exposure locations, and/or a trend between locations that are exposed to different degrees of contamination. The hypotheses address either the ability of a particular monitoring tool to detect such an effect (and, in aggregate, whether an effect exists), or the **relative** ability of two different monitoring tools, that are being compared to one another, to detect such an effect. H5 through H8 are of the first type, while H1 through H4 are of the second type. H9 through H12 address the integration of tools and the **relative** ability of two monitoring tools to detect a correlation between specific exposure and response variables, while H13 addresses the ability of a particular toxicity testing tool to show such a correlation.

These different types of hypotheses require different methods of statistical analysis. The following sub-sections describe the statistical approach in each category. In all cases, appropriate data transformations were applied prior to statistical analysis, such as log transformation for chemical concentrations, or other parameters that span a wide range, and arcsine square root transformations for percent response variables. A significance criterion of $p \leq 0.05$ was used for all the statistical analyses, and use of the term "significant" implies that this criterion was met.

It should be recognized that the term "predictor" variable is not intended to mean that the measure of exposure used (e.g., metal concentration in water) can be used to "predict" a specific biological response at all mine sites or in other surveys at this mine site. Nor does it imply that the predictor is necessarily the cause of a biological effect. Rather, the predictive ability is only **suggested** by correlation between effect and exposure measures.

### 5.1.1 H4 - Metal vs Metallothionein in Tissue

Hypothesis H4 addresses the **relative** ability of two monitoring tools (response measures) to detect a mine effect (i.e., metals in fish tissues versus metallothionein in fish tissues). In particular, metallothionein in fish viscera was compared to each of the individual metals in fish viscera, to

## TABLE 5.1: VARIABLES AND HYPOTHESES AT HEATH STEELE

| Hypothesis | Response or Effect Variables (Y) | Predictor or Exposure Variables (X) | Null Hypothesis | Comment |
|---|---|---|---|---|
| H4 | Metal i in Tissue j (Tool 1)<br>MT in Tissue j (Tool 2) | Reach Number (in order of increasing dilution downstream from mine) | no trend or R/E x tool interaction by ANOVA | For blacknose dace, juvenile salmon. Repeat with caged salmon. Viscera only. Partial results presented for gill (caged salmon) and brook trout (viscera) also. |
| H5 | CPUE for dace, juvenile salmon, all species | Reach Number (in order of increasing dilution downstream) | no trend or R/E difference by ANOVA | CPUE for salmon tested using data for comparable stations (equal barrier effects) |
| H6 | BPUE (biomass) for fish<br>No. of Taxa (fish, benthos, periphyton)<br>EPT Taxa<br>Benthic Density | Reach Number (in order of increasing dilution downstream from mine) | no trend or R/E difference by ANOVA | Collections at several stations per reach |
| H7 | Weight at age<br>Length at age | Reach Number (in order of increasing dilution downstream) | no trend or R/E difference by ANOVA | Mature minnows and small salmon<br>Use age covariate as appropriate |
| H9 | Benthic Density<br>No. of Benthic Taxa<br>EPT Index<br>Fish CPUE and BPUE<br>No. of Fish Taxa<br>Periphyton Community Indices<br>Effluent Chronic Toxicity | Dissolved Metal in Water (Tool 1)<br>Total Metal in Water (Tool 2) | same Y-X correlation with Tool 1 as Tool 2 | May be other benthic indices, as revealed by multivariate analysis |
| H10 | Benthic Density<br>No. of Benthic Taxa<br>EPT Index<br>Fish CPUE and BPUE<br>No. of Fish Taxa | Fraction Metal i in Periphyton (Tool 1)<br>Dissolved Metal i in Water (Tool 2) | same Y-X correlation with Tool 1 as Tool 2 | Use periphyton for "sediment";<br>Dissolved metals used in comparison. Dissolved metals and total metals were similarly correlated with benthic responses in H9. |
| H12 | Metal i in Tissue j<br>MT in Tissue j | Metal i in Periphyton (Tool 1)<br>Dissolved Metal i in Water (Tool 2) | same Y-X correlation with Tool 1 as Tool 2 | Use periphyton for "sediment" |
| H13 | Benthic Density<br>No. of Benthic Taxa<br>EPT Index<br>Fish CPUE and BPUE<br>No. of Fish Taxa | Calculated % Inhibition in Exposure Reach | no Y-X correlation | Calculated % inhibition *in situ* based on effluent toxicity tests and water/effluent sulphate concentration ratio |

Definitions:
MT = metallothionein
R/E = reference/exposure
CPUE = catch-per-unit-effort (number of fish caught per unit fishing effort)
BPUE = biomass-per-unit-effort (mass of fish caught per unit fishing effort)
EPT taxa = Ephemeroptera (mayflies), Plecoptera (stoneflies) and Trichoptera (caddisflies) (pollution-sensitive benthic invertebrates)

determine whether these two monitoring tools differ in their ability to detect a mine effect (i.e., a reference vs exposure area difference, or a trend with degree of exposure within the exposure area). A stream reach identifier (e.g., HE1, HE2, etc.), ordered within the exposure area to reflect distance from the mine site, was used as a surrogate for exposure to mine effluents because, as distance from the mine increased, so did dilution of the effluent. Figure 2.3 illustrates the reach identifiers. Analysis of variance (ANOVA) was used to address this hypothesis, as described below. Essentially, the ANOVA is used to compare tools in two ways:

- by determining if there is a reference area - exposure area difference in mean values for each tool (a larger difference indicates greater effectiveness in detecting an effect with this data set); and
- by determining if there is a linear trend or gradient in response within the exposure area (a significant trend and greater slope indicates greater effectiveness in detecting an effect with this data set).

The ANOVA partitions overall variance in the response measure into a number of terms, representing effects of particular interest. These include:

- A "Ref vs Exp x Tool" term which indicates whether the Reference versus Exposure difference is similar for both tools (e.g., for metallothionein and copper in tissue). It measures how much the spread between Line 1 and Line 2 differs from the spread between Line 3 and Line 4 in Figure 5.1. Lines 1 to 4 represent the means of the response measures for each tool in the reference or exposure area. This term also indicates how much the Line 1 to Line 3 spread differs from the Line 2 to Line 4 spread, or the degree of difference between the slopes of the two lines shown in Figure 5.2. A larger difference between the reference and exposure means for one tool relative to the other would indicate a greater effectiveness for the tool with the greater difference. For this example, the absolute reference-exposure difference for each tool is small, but the differences are in opposite directions. This produces a significant Ref vs Exp x Tool interaction, which implies that Tool 1 (metallothionein) is more effective than Tool 2 (copper in viscera). The interaction is also illustrated in Figure 5.2.
- A "Linear Trend x Tool" term which indicates whether the linear trend in the Exposure area (e.g., from near-field to far-field) is similar for both tools. It measures how much the Line 2 to Line 5 spread differs from the Line 4 to Line 6

spread in Figure 5.1. This term also indicates the degree of difference between the Line 5 and Line 6 slopes. A greater slope in the Line 5 (Tool 1) than in Line 6 (Tool 2) indicates a greater effectiveness of Tool 1 in this example.

In all cases, to test whether the spread described in either of the above two "effect" terms is significant, each is compared to the spread of the exposure means for each reach around Lines 5 and 6 (i.e., to a lack of fit "error" term). If the "effect" variance is large relative to the "error" variance, then the effect is considered to be present, and the tool is concluded to be responsive to mine exposure.

The "lack of fit" spread is compared in turn to the overall "within reach" spread (i.e., between stations in a particular reach), in order to test whether there may be any other (i.e., non-linear) trend among the exposure means, that is whether a straight line can be drawn through response measures for all exposure reaches. If "lack of fit" is significant, the nature of the trend is examined and, if appropriate, the analysis is repeated using a non-linear (second order) trend term instead of a linear trend term. This would appear in Figure 5.1 as curved lines rather than the straight Lines 5 and 6.

The response measures for H4 (metal or metallothionein in fish tissue) were standardized prior to statistical analysis, in order to make them equally variable within a reach, since homogeneity of variance is an assumption of the ANOVA procedure. The standardization procedure involves dividing the metal values by the pooled within-reach standard deviation for the metal being evaluated, and dividing the metallothionein values by the pooled within-reach standard deviation for metallothionein.

### 5.1.2 H5 through H7 - Fish CPUE, Community Structure and Fish Growth

Hypotheses H5 through H7 address the ability of a particular monitoring tool (response measure) to detect a mine effect. For example, in H5, fish catch-per-unit-effort (CPUE) was compared across reaches to determine whether it demonstrates a mine effect (i.e., a reference vs exposure area difference), or a trend with degree of exposure within the exposure area. A reach identifier, ordered within the exposure area to reflect distance from the mine site, was used as a surrogate for exposure to mine effluents. Analysis of variance (ANOVA) was used to address this hypothesis, as described below.

The ANOVA partitions overall variance in the response measure into a number of terms, representing effects of particular interest. These include:

- An "Among Reference" term which indicates whether the various Reference reaches are similar to each other. It measures the spread of reference means around Line 1 in Figure 5.3 (i.e., around the grand reference mean represented by the solid line). This term is quantified in order to indicate whether reference reaches are differentially influenced by some factor (e.g., habitat) that may also be confounding effects in the exposure area.
- A "Ref vs Exp" term which indicates whether the Reference and Exposure reaches are similar to each other. It measures the spread between Line 1 (reference mean) and Line 2 (exposure mean) in Figure 5.3 (i.e., between reference and exposure means). A reference-exposure difference is indicative of tool effectiveness, assuming that the direction of the difference is consistent with impact.
- A "Linear Trend" term which indicates whether there is a linear trend in the Exposure area (e.g., from near-field to far-field). It measures the spread between Line 2 and Line 3 (the exposure trend line) in Figure 5.3 (i.e., the difference in slopes). A significant linear trend, i.e., a near-field to far-field gradient is indicative of tool effectiveness, assuming that its direction is consistent with impact.

In all cases, to test whether the spread is significant, as described in any of the above three "effect" terms, each is compared to the spread of exposure reach means around Line 3 (i.e., to a "lack of fit" error term). This "lack of fit" error term accounts for the residual variability in the data after the above three terms are subtracted from the total among-reach variability. If an "effect" term is large relative to the "lack of fit" error, then the effect is more likely to be significant.

The "lack of fit" spread is compared in turn to the overall "within reach" spread (i.e., between stations within a reach), in order to test whether there may be any other (i.e., non-linear) trend among the exposure means, that is whether a straight line is the best description of the trend. If "lack of fit" is significant, the nature of the trend is examined and, if appropriate, the analysis is repeated using a non-linear (second order) trend term instead of a

linear trend term. This would appear in Figure 5.3 as a curved line rather than straight Line 3.

In the example, the data points in Figure 5.3 represent CPUE at each station for all fish species. The ANOVA shows a significant "Ref vs Exp" effect, because there is a substantial difference between Lines 1 and 2. The ANOVA also shows that there is a significant "Linear Trend" effect, because CPUE is lowest near the mine (Reach HE1) and increases as we move further away (i.e., slope of Line 3). The interpretation would be that fish abundance is responding to mine exposure.

H6, which is intended to identify fish community tools, has been expanded in the case of Heath Steele to include benthic community tools. This is appropriate, because subsequent hypotheses (H9 and H10) involve benthic as well as fish community tools and their chemical correlations. Community tools which seem to reflect mine effects are of particular interest. However, benthic community response tools tested, in H6, include only a few biotic indices in common use or showing apparent response to mine exposure. The evaluation is not extended to the point where the multitude of diversity and biotic indices available are evaluated in terms of exposure response. H6 is also tested using periphyton community tools.

For H7, the response measure (fish weight or length) varies with fish age. Therefore, an age covariate was added to the ANOVA model in order to adjust all fish to a common age. The statistical analysis of age-adjusted data is as described above.

Such age adjustment is inappropriate when the form (i.e., slope) of the size-age relationship differs among reaches. This was true for Atlantic salmon when young-of-year (YOY) fish and all reaches were included in the analysis. The YOY were **smaller** in size in areas of high YOY density (e.g., reach HR3). Therefore, YOY were excluded from the analysis in order to perform the age adjustment and test for other growth effects (i.e., on the intercept of the size-age relationship) using ANOVA as described above.

Reaches HR3 and HE5 were excluded from analyses involving salmon density (H5), because they are unaffected by a barrier that limits the spawning run at points upstream, and are therefore not comparable to the upstream study area. A similar exclusion is probably appropriate for analyses involving salmon growth (H7); however, this leaves only three reaches (HR2, HE3, HE4) and two among-reach degrees of freedom, which is insufficient to

support the partitioning of among-reach variance that is described above. Therefore, analysis of H7 for salmon was performed with and without the exclusion of reaches HE3 and HE5.

### 5.1.3 H9 through H12 - Tool Integration Hypotheses

Hypotheses H9, H10 and H12 address the **relative** ability of two monitoring tools to detect correlation between exposure and response variables. For example, in H9, dissolved metal in water was compared to total metal in water, for each of the key metals, to determine whether these two monitoring tools differ in their ability to detect a mine effect (i.e., a correlation between a biological response measure, such as number of taxa, and the metal exposure variable). Correlation analysis was used to address this hypothesis, as described below.

The squared coefficient of correlation ($r^2$) between the response measure (Y) and each exposure variable (X1 or X2) indicates the proportion of variance in the response measure that is explained by the predictor (Figure 5.4). The best predictor for each pair compared is the one which explains the highest proportion of variance (i.e., has the highest $r^2$ and hence the highest r). No statistical test was performed to determine whether $r_1$ differs significantly from $r_2$, since the two r values are based on the same Y data set and are not independent. However, the individual r values were tested for statistical significance. Two r values were compared, to draw inferences about which monitoring tool is better, only when at least one of the r values was of the correct sign (negative or positive) to suggest a mine effect, and statistically distinguishable from zero based on a one-tailed test.

These correlations were computed excluding reference stations. Response tools correlated with potential causal agents when reference sites are excluded are considered more effective than those showing correlations only when reference sites are included. This is because correlations seen within the exposure gradient are more clearly associated with mine impact. The inclusion of data from up to three reference site reaches could potentially impose spurious correlations by producing clusters of data points at low exposure concentrations. It must be noted that the decision to compute correlations excluding references stations is not supported by scientists working on metal toxicology and MT in fish who believed that important biological information are lost using this procedure.

In the example shown in Figure 5.4, dissolved lead is a more effective predictor of numbers of benthic taxa in exposure reaches than is total lead.

When differences between r values are small (e.g., ≤0.1), even though one or both r values may be statistically significant, a judgement is generally not made that the tool with the slightly higher r value is better able to detect an effect. Also, the correlations are generally calculated for many exposure measures (metals), so that judgements with respect to which exposure measure tool (e.g., total versus dissolved concentration in water) is more strongly correlated with biological response are made by the weight-of-evidence based on all r values for each tool. The exposure and response measures selected for inclusion in this analysis were those which showed an apparent spatial relationship to the mine site, i.e., trend among exposure reaches or difference between reference and exposure reaches.

At Heath Steele, H9 (relationship between water quality and biological variables) is tested both using benthic community tools and fish community tools. This hypothesis compares the strength of correlations of dissolved versus total metals in water with biological responses.

H10 (relationship between sediment chemistry and biological tools) is tested using periphyton metal concentration as a surrogate for sediment chemistry. Because H10 compares different sediment chemistry tools (e.g., total versus partial sediment metals) and only one "sediment" chemistry tool is available here (total metals in periphyton), H10 is tested by comparison of periphyton metals and dissolved metals.

H12 (relationship between water and sediment chemistry and fish tissue chemistry response) is tested using dissolved water chemistry and periphyton chemistry in the environment, and metallothionein and metals in fish viscera.

### 5.1.4 H13 - Chronic Toxicity Linkage with Benthic and Fish Community Results

Hypothesis H13 addresses the ability of a particular effluent toxicity testing tool to predict a mine effect that has been otherwise demonstrated (e.g., in H5 to H7). For example, H13 might address whether fish catch-per-unit-effort (CPUE) in each downstream reach can be predicted from effluent toxicity to *Ceriodaphnia*. In order to test this hypothesis, it is necessary to estimate the receiving water toxicity to *Ceriodaphnia* in each reach, based on the effluent toxicity information and the expected downstream dilution of effluent. Then we can determine if these two reach attributes (fish CPUE and water toxicity) are correlated as they vary from reach to reach.

Water toxicity, like effluent toxicity, can be expressed as a % inhibition (i.e., for *Ceriodaphnia* as % inhibition of reproduction). The % inhibition increases with effluent concentration. The IC25 concentration produces 25% inhibition, and the IC50 concentration produces 50% inhibition. These two concentrations, obtained from the effluent toxicity test, define the % inhibition vs concentration relationship. We can use this relationship to estimate the % inhibition that would be expected at each effluent concentration that exists in the downstream reaches.

The % inhibition vs concentration relationship has a sigmoid form, such that % inhibition increases most rapidly with concentration in the vicinity of the IC50 concentration (Figure 5.5). It is standard practice to transform both variables (i.e., % inhibition and effluent concentration) to make a linear relationship, in order to facilitate estimation of % inhibition at any concentration. A probit (or Z) transformation of % inhibition and a log transformation of effluent concentration will accomplish this (Finney, 1971). Figure 5.6 illustrates the linearized relationship, based on the *Ceriodaphnia* IC25 and IC50 concentrations for the June "effluent" sample at Heath Steele. It also illustrates the use of the relationship to estimate water toxicity (% inhibition of *Ceriodaphnia* reproduction) at reach HE2 downstream.

Water toxicity was estimated in this manner for each reach downstream of the mine, based on three different effluent samples (June, August, November) and up to four different toxicity test methods (*Ceriodaphnia*, fathead minnow, algae, duckweed). It can only be done for tests that produce both IC25 and IC50 values (i.e., two points are necessary to draw a concentration-response line in Figure 5.6). Two minnow tests and one duckweed test at Heath Steele did not produce both endpoints. Thus, there were nine different water toxicity variables (i.e., different estimators of % inhibition). Each of these toxicity variables was tested for correlation with each of the field measurements of biological response, such as fish CPUE, and plots such as Figure 5.7 were produced to illustrate some of the stronger relationships.

Appropriate transformations were applied prior to the correlation analysis. For example, the arcsine square root of % inhibition was used as the water toxicity variable, and fish CPUE was log transformed. Then the correlation coefficient (r) was computed and tested for significance using a one-tailed t-test. Significance depends only on the magnitude of r and a sample size (n). For n=5 exposure reaches, r must be greater than 0.81 (i.e., $r^2 > 0.65$) to produce a significant correlation. In Figure 5.7, the November effluent sample produces the

strongest CPUE vs water toxicity relationship ($r^2 = 0.76$), but all the relationships shown are significant.

A significant correlation (r) indicates that the toxicity tool may be useful as a predictor of the in-stream biological response measure. It does not, of course, prove that effluent is responsible for any observed pattern in biological response downstream from the mine. The toxicity test methods that generally provide the highest correlations with biological response measures are considered to be the best.

An estimate of % inhibition in the downstream reach (as described above) is likely to be a better predictor of biological response than a simple toxic unit (TU) predictor. The former uses the concentration-response information obtained from the toxicity test, while TU is simply a dilution factor for the reach scaled by the IC25 (or IC50) concentration of the effluent. As such, a TU predictor would show exactly the same relationship to biological response as the dilution factor, and would not effectively utilize the exposure-response information from the toxicity test, as given in Figure 5.5. In other words, the predicted % inhibition approach used, unlike a TU approach, incorporates information on whether there is a large or small change in toxicity with a specified change in effluent concentration.

Using the % inhibition approach, if there is a biological response downstream from the mine, and if there is sufficient dilution relative to effluent toxicity that zero % inhibition is expected at all downstream locations, then the points in Figure 5.7 will fall in a vertical line and the correlation will not be significant.

## 5.2 Results

The general conclusions with respect to the eight hypotheses tested at Heath Steele are summarized in Table 5.2. The following sections present the findings in more detail, based on statistical tables and figures in Appendix 3. The discussion is focused on results that meet the significance criterion of $p \leq 0.05$. Use of the term "significant" implies that this criterion was met, although "suggested" results may be mentioned as such when the criterion is approached but not achieved.

### 5.2.1 H4 - Metallothionein vs Metal in Fish Tissue as a Response to Exposure

Figures illustrating the response patterns of metallothionein and metals in fish tissue, and ANOVA tables showing tests for differences in response patterns between metallothionein

## TABLE 5.2: SUMMARY OF GENERAL CONCLUSIONS REGARDING HYPOTHESES TESTED AT HEATH STEELE

| Hypothesis | Response or Effect Variables (Y) | Predictor or Exposure Variables (X) | Null Hypothesis | General Conclusion |
|---|---|---|---|---|
| H4 | Metal i in Tissue j (Tool 1)<br>MT in Tissue j (Tool 2) | Reach Number (in order of increasing dilution downstream from mine) | no trend or R/E x tool interaction by ANOVA | • MT vs metals (Cd, Pb, Zn, Cu) show different trends in the exposure area (MT trend stronger) for caged salmon.<br>• MT vs metals (Cd, Pb, Zn, Cu) are similar in their lack of trend in the exposure area for wild salmon.<br>• MT vs metals (Cd, Pb, Zn) show different trends in the exposure area (MT trend stronger) for blacknose dace.<br>• MT and Cu change in opposite directions from Reference to Exposure areas (MT higher in Exposure area) for caged salmon.<br>• MT vs metals (Cd, Pb, Zn) show different degrees of change from Reference to Exposure areas (both higher in Exposure area) for blacknose dace. |
| H5 | CPUE for dace, juvenile salmon, all species | Reach Number (in order of increasing dilution downstream from mine) | no trend or R/E difference by ANOVA | • Fish CPUE (all taxa combined) is reduced with degree of exposure and E<R mean. |
| H6 | BPUE (biomass) for Fish<br><br>No. of Benthic Taxa and EPT Taxa<br><br><br><br>Total Benthic Density<br>% Chironomid Indicators<br>- % Rheocricotopus<br>- % Orthocladoinae<br><br>Periphyton Taxa and Biomass | Reach Number (in order of increasing dilution downstream from mine) | no trend or R/E difference by ANOVA | • Fish BPUE (all taxa combined) is reduced with degree of exposure and E<R mean.<br>• E<R mean for number of benthic taxa; no linear trend in exposure zone.<br>• Number of EPT taxa is reduced with exposure and E<R mean.<br>• No spatial trends evident in total density.<br>• Rheocricotopus dominance showed a linear trend in exposure area and a E/R mean difference.<br>• Orthoclad dominance showed a trend in the exposure area.<br>• Periphyton community indices showed no spatial trends. |
| H7 | Weight at age<br>Length at age | Reach Number (in order of increasing dilution downstream from mine) | no trend or R/E difference by ANOVA | • YOY salmon are smaller in high density reaches (below barriers).<br>• Effect persists at later ages.<br>• No impairment of growth by exposure for salmon or blacknose dace. |
| | | | | • |

## TABLE 5.2: SUMMARY OF GENERAL CONCLUSIONS REGARDING HYPOTHESES TESTED AT HEATH STEELE

| Hypothesis | Response or Effect Variables (Y) | Predictor or Exposure Variables (X) | Null Hypothesis | General Conclusion |
|---|---|---|---|---|
| H9 | No. of Benthic Taxa<br>EPT Index<br>% Chironomid Indicators<br><br>Fish CPUE and BPUE<br>No. of Fish Taxa<br>Predicted In-stream Toxicity | Dissolved Metal in Water (Tool 1)<br>Total Metal in Water (Tool 2) | same Y-X correlation with Tool 1 as Tool 2 | • Numbers of total benthic taxa and EPT taxa are reduced and dominance of tolerant chironomids increases with increasing metal in water.<br>• Fish CPUE, BPUE and number of fish taxa decrease with increasing metal in water.<br>• Relationships similar for dissolved and total metals.<br>• Relationships similar for dissolved and total metals. |
| H10 | No. of Benthic Taxa<br>EPT Index<br>% Chironomid Indicators<br><br>Fish CPUE and BPUE<br>No. of Fish Taxa<br><br><br>Predicted In-stream Toxicity | Fraction Metal i in Periphyton (Tool 1)<br>Dissolved Metal i in Water (Tool 2) | same Y-X correlation with Tool 1 as Tool 2 | • Numbers of total benthic taxa and EPT taxa are reduced and dominance of tolerant chironomids increases with increasing metals in periphyton and water.<br>• Fish CPUE, BPUE and number of taxa decrease with increasing metals in periphyton and water.<br>• For most fish and benthic indices, relationships stronger with metals in water than metals in periphyton.<br>• Relationships slightly stronger for Al, Cd, Cu and Zn in water and for Pb and Fe in periphyton. |
| H12 | Metal i in Tissue j<br>MT in Tissue j | Metal i in Periphyton (Tool 1)<br>Dissolved Metal i in Water (Tool 2) | same Y-X correlation with Tool 1 as Tool 2 | • MT in wild and caged salmon viscera increases with metals in water; metals in salmon did not increase with metals in water or periphyton.<br>• MT in blacknose dace viscera increases with Pb in water; Zn, Cu and Pb in dace viscera increase with metals in periphyton. |
| H13 | Fish CPUE and BPUE<br>% Chironomid Indicators<br>No. of Benthic Taxa<br>EPT Index<br>No. of Fish Taxa | Predicted % Inhibition in Exposure Reach based on effluent toxicity testing and downstream dilution factors | no Y-X correlation | • Fish CPUE, BPUE and number of taxa decrease with predicted water toxicity to algae, *Ceriodaphnia*, duckweed or fathead minnow.<br>• Dominance of pollution-tolerant chironomids increases with predicted water toxicity. Other benthic indices not correlated with predicted toxicity.<br>• The four toxicity tests produce similar biology vs predicted toxicity correlations, when effluent is sublethally toxic.<br>• Fathead minnow test is less sensitive than other tests. |

**TABLE 5.2: SUMMARY OF GENERAL CONCLUSIONS REGARDING HYPOTHESES TESTED AT HEATH STEELE**

E - exposure reaches
R - reference reaches.

and metals are provided in Appendix 3. Based on these patterns and statistical test results, the key findings regarding Hypothesis H4 are outlined below, for caged Atlantic salmon (viscera), wild Atlantic salmon (viscera) and wild blacknose dace (viscera). This hypothesis was addressed only for viscera because quantities of other tissues were insufficient for both metallothionein and metal analyses.

**Caged Atlantic Salmon (Viscera)**

Metallothionein (log concentration in tissue) shows an increasing trend with increasing exposure within the exposure area (i.e., from HE5 in the North Branch Tomogonops, upstream to HE1 in the Little South Branch) ($p = 0.009$). Similarly, the exposure area mean level of metallothionein is somewhat elevated relative to the reference mean (HR1 to HR3), although this result is not significant ($p = 0.09$).

Metals in fish tissue (log concentration of Zn, Pb, Cd, Cu) do not show a trend in the exposure area. Similarly, the exposure area mean levels of metals in tissue are not elevated relative to the reference mean. In the case of Cu, the reference area mean exceeds the exposure area mean, in contrast to the direction of difference for metallothionein.

The trend in metallothionein, but not metals, results in a significant trend x tool interaction ($p < 0.05$) with Zn, Pb, Cd, Cu and the molar sum of Cd+Cu+Zn. In addition, the difference in exposure versus reference mean for Cu, which was in the opposite direction as compared to metallothionein, resulted in a significant reference/exposure x tool interaction ($p = 0.015$).

**Wild Atlantic Salmon (Viscera)**

In wild Atlantic salmon, metallothionein (log concentration in tissue) does not show an increasing trend with increasing exposure level within the exposure area (i.e., from HE5 upstream to HE3). The exposure area mean suggests an elevation relative to the reference area mean (HR2 and HR3); however, this difference is not significant ($p = 0.099$).

Some metals in fish tissue show a similar response pattern, with no trend in the exposure area, but a small elevation in the exposure area mean. The difference is significant ($p = 0.019$) for log concentration of Pb in tissue. Other metals, such as Zn, show no elevation in the exposure area. No metals show a significant spatial trend in the exposure area.

The metallothionein and metal tools were equally effective (or ineffective) in wild Atlantic salmon at Heath Steele, as indicated by the lack of any significant reference vs exposure x tool interactions. However, our ability to statistically detect such interactions was limited by availability of Atlantic salmon data for only three exposure reaches and two reference reaches.

It appears that there may be a weak metallothionein response in the HE5 to HE3 region (there were no salmon caught closer to the mine), as indicated by the elevated levels in this exposure area (although not significant). However, in contrast to the caged salmon, no spatial trend is evident, in either metallothionein or metal, within this far-field area. This difference as compared to caged fish may be related to the longer exposure period of wild fish (wild fish in this area had about eight times as much metallothionein and about two times as much metal as the caged fish) or to the greater mobility of wild fish. This mobility would lead to spatial averaging across the exposure area.

**Wild Blacknose Dace (Viscera)**

Blacknose dace show a response pattern similar to caged Atlantic salmon. In this case, the suggested metallothionein trend within the exposure area is not significant ($p = 0.06$), but the elevation in the exposure area mean level of metallothionein relative to the reference area mean is significant ($p = 0.008$).

There is an elevated level of some metals (log concentration of Zn and Cu) in the tissues of dace from the exposure area. Other metals, such as Pb, are not elevated in the exposure area. No metals show a significant spatial trend in the exposure area ($p > 0.05$).

The trend in metallothionein, but not metals, results in a significant trend x tool interaction for Zn ($p = 0.046$), Pb ($p = 0.049$) and Cd ($p = 0.002$). In addition, there is a significant reference vs exposure x tool interaction ($p < 0.05$) for these metals, indicating that the reference-exposure difference for metallothionein is greater or smaller than it is for metals (depending on the metal).

The metallothionein trend seen in caged salmon and blacknose dace, but not wild salmon, suggests that the lack of trend in wild salmon may be related to their mobility. The exposure area elevation of some metals, seen in wild salmon (Pb) and wild dace (Cu, Zn), suggests that the short exposure period may prevent detection of a similar effect in caged fish.

### 5.2.2 H5 through H7

#### *5.2.2.1 H5 - Fish CPUE as a Response to Exposure*

Figures illustrating the response patterns of fish CPUE in relation to mine exposure, and ANOVA tables showing tests for significance of these trends, are provided in Appendix 3. Based on these patterns and statistical test results, the key findings regarding hypothesis H5 are outlined below for Atlantic salmon, blacknose dace, brook trout and the overall fish community.

**Atlantic Salmon CPUE**

The analysis for Atlantic salmon was confined to catch at HE1 to HE4 and HR2, because these reaches were all influenced by the same barrier, i.e., the railway bridge on the North Branch Tomogonops River. Salmon are excluded from HR1, upstream of the mine, by barriers downstream of HR1.

Catch was zero at HE1 and HE2 nearest the mine, and increased at HE3 and HE4 further downstream. However, the suggested trend in log CPUE within the exposure area was non-significant ($p = 0.069$).

The exposure area mean, although elevated relative to HR2, was not significantly different than the reference mean. It should be noted that the reference mean in this case is represented by a single reach with two stations.

**Blacknose Dace CPUE**

There was no demonstrable trend of increasing catch in the downstream direction, nor any difference between exposure and reference areas. Reach HE4 produced a very low catch, contrary to what may have been a trend otherwise.

Unknown habitat effects may confound the use of this tool, as suggested also by a discontinuity in benthic community measurements in HE4. There was considerable variability among reference reaches.

**Brook Trout CPUE**

There was no demonstrable trend of increasing catch in the downstream direction, nor any difference between exposure and reference areas.

Mine effects may be confounded by habitat effects since HE4 and HE5 in the North Branch Tomogonops River are probably sub-optimal for brook trout based on the river size and flow.

**Fish Community CPUE**

The suggested trend of increasing catch of all fish in the downstream direction was statistically significant ($p = 0.043$), and the exposure area mean CPUE was significantly reduced relative to the reference mean ($p = 0.029$). Consequently, this tool was useful in detecting mine effects on the fish community at Heath Steele. The greater effectiveness of this tool is attributed to the dampening of "noise" in the relationships when all species are included, including not only the three listed but others such as lake chub which were also present.

### *5.2.2.2 H6 - Biological Community Measures as a Response to Exposure*

Figures illustrating the response patterns of fish biomass and benthic community measures in relation to mine exposure, and ANOVA tables showing tests of significance for these trends, are provided in Appendix 3. Based on these patterns and statistical test results, the key findings regarding hypothesis H6 are outlined below.

**FISH**

**Atlantic Salmon BPUE**

The suggested trend of increasing biomass in the downstream direction was not statistically significant ($p = 0.074$), and the exposure area mean was not significantly elevated relative to the reference mean.

**Blacknose Dace BPUE**

There was no demonstrable trend of increasing biomass in the downstream direction, nor any difference between exposure and reference areas. Reach HE4 produced a very low biomass, contrary to what may have been a trend otherwise.

Unknown habitat effects at HE4 may confound the use of this tool, as suggested also by a discontinuity in benthic community measurements at HE4. There was considerable variability among reference reaches.

### Brook Trout BPUE

The apparent linear trend of increasing biomass in the downstream direction was not statistically significant ($p = 0.206$; however, a second order trend was significant. In other words, biomass changed more rapidly with distance in the near-field than in the far-field exposure area. Using this second order trend to improve the fit of the trend model, the lower biomass in the exposure area, as compared to reference, was significant ($p = 0.023$). Consequently, brook trout BPUE was useful in detecting mine effects at Heath Steele.

Mine effects may be confounded by habitat effects, since HE4 and HE5 in the North Branch Tomogonops River are probably sub-optimal for brook trout based on the river size and flow.

### Fish Community BPUE

The linear trend of increasing biomass in the downstream direction was statistically significant ($p = 0.02$). However, a second order trend fit the data better, suggesting that there is less change with distance in areas that are further away from the mine site. Using the second order term to describe the trend, it was shown that the exposure area mean BPUE was significantly reduced relative to the reference mean ($p = 0.045$). Consequently, fish BPUE was useful in detecting mine effects on the fish community at Heath Steele.

## BENTHIC INVERTEBRATES

### Number of Benthic Taxa

The mean number of benthic taxa was significantly reduced in the exposure area relative to the reference area. This is because metal-tolerant species tend to replace more sensitive species in the exposure areas.

The suggested trend of increasing species richness in the downstream direction was not statistically significant, in particular due to the low species richness at HE4. An unknown habitat effect may be involved here.

### Number of EPT Taxa

The linear trend of increasing EPT taxa (mayflies, stoneflies and caddisflies) in the downstream direction was statistically significant ($p = 0.029$), and the exposure area mean was significantly reduced relative to the reference area mean ($p = 0.023$). The EPT taxa are generally sensitive to pollution and are considered to be indicators of good water and sediment quality. Thus, they may be considered useful in detecting effects on the benthic community at Heath Steele.

### Rheocricotopus (Dominance)

The dominance of the chironomid *Rheocricotopus* (arcsine square root transformed % total benthic density) decreased significantly in the downstream direction ($p = 0.025$), and the exposure area mean was significantly greater than the reference mean ($p = 0.047$). This genus is apparently pollution-tolerant and does well in the most exposed reaches of the Little South Branch. Thus, it may be considered useful as an indicator of effects at Heath Steele.

### Orthocladiinae (Dominance)

The dominance of this sub-family of chironomids (arcsine square root transformed % total benthic density) decreased significantly in the downstream direction ($p = 0.012$), mainly in the near-field (HE1 to HE3). The exposure area mean was not greater than the reference area mean (0.052). Like *Rheocricotopus*, this group is pollution-tolerant and is more dominant near the mine. Thus, it may be considered useful as an indicator of mine effects at Heath Steele.

## PERIPHYTON

### Number of Periphyton Taxa

The number of periphyton taxa did not differ significantly among reaches. Therefore, no tests for spatial trend or comparisons between areas were performed.

**Periphyton Biomass**

The log of periphyton biomass showed no significant spatial trend or difference between reference and exposure areas.

### *5.2.2.3 H7 - Fish Growth as a Response to Exposure*

Figures illustrating the size-age relationships of Atlantic salmon and blacknose dace, as well as age-adjusted weights in relation to mine exposure, and ANOVA tables showing tests of significance for these trends, are provided in Appendix 3. Based on these patterns and statistical test results, the key findings regarding hypothesis H7 are outlined below.

**Atlantic Salmon**

Atlantic salmon tend to be larger at age at more exposed locations HE3 and HE4 than at less exposed sites (HE5) or at reference reach HR3. This is not consistent with an expected metal exposure effect, and may be associated with a density-dependent growth response where growth is reduced in reaches where salmon density is high.

Young-of-year (YOY) fish are smaller in high density reaches (HR3, HE5), which produces different slopes in the size-age relationships for different reaches. If we exclude the young-of-the-year (YOY) fish, the slopes are the same and we can adjust for age to examine other possible effects on fish size. After we do this, there is still an effect of reach on size at age (i.e., on the intercept of the size-age relationship) as indicated by small size-at-age at HR3 and to a lesser extent at HE5. Thus, the apparent density-dependent effects on young fish growth at these stations remain evident in older fish. Both these stations are unaffected by the barrier (railway bridge) that limits the spawning run to points upstream.

If we leave HR3 and HE5 stations in the analysis, there are significant among-reach effects on fork length ($p = 0.023$ based on station mean values), but no discernible reference-exposure difference, and no discernible trend within the exposure area, for either fork length or weight. If we exclude these stations from the analysis, there is no significant among-reach difference.

**Blacknose Dace**

For statistical analyses involving fork length, only fish ≤3 years of age were used because the presence of older fish (which grow slowly) in some reaches produced a significant length x age interaction. For analyses of fish weight or weight-at-age, the interaction was not significant, so all fish were utilized. Adjusting for the age effects, neither length-at-age or weight-at-age differed among reaches.

### 5.2.3 H9 through H12

These hypotheses involve examination of correlation coefficients between measured parameters. The correlations can be computed in two ways: excluding and including the reference stations. We consider it more appropriate to exclude the reference stations in the hypothesis testing, so that the correlations clearly reflect relationships that exist within the mine exposure gradient, rather than extreme values on the X-axis driven by three reference reaches (six reference stations). Thus, a total that produces a high correlation coefficient when tested with exposure station data only is more effective than one producing high values only when reference site data are included in the analysis. Only the results generated exclusive of reference station data are discussed in this report. While no statistical tests were performed to compare the correlations generated by two measurement tools, differences of about 0.1 or more between coefficients are considered worthy of discussion, as long as at least one of the coefficients is statistically distinguishable from zero.

#### *5.2.3.1 H9 - Correlation of Biological Response with Dissolved vs Total Metal in Water*

Tables showing the correlation coefficients between water chemical and biological measurements are provided in Appendix 3. Based on the magnitudes of the significant correlation coefficients, the key findings regarding hypothesis H9 are outlined below.

**Correlation of Community Structure with Dissolvedvs Total Metal in Water**

These correlations are negative for fish CPUE and BPUE (slightly stronger for BPUE in general) and for number of fish taxa. In other words, CPUE, BPUE and fish taxa tend to

decrease with increasing metal concentrations. These CPUE and BPUE correlations are strongest for the fish community as a whole (all species) and for Atlantic salmon, which showed significant ($p < 0.05$) or near significant ($0.05 < p < 0.1$) responses in H5 and H6.

The correlations are negative for number of benthic taxa and EPT taxa, but positive for dominance of pollution-tolerant taxa such as *Rheocricotopus* and Orthocladiinae. In other words, as metal concentrations increase, the number of taxa decreases and pollution-tolerant species comprise a greater percentage of total organism density. These results are generally consistent with the benthic response trends seen in H6.

On balance, the strength of correlations between fish or benthic response and aqueous metal concentrations are similar for dissolved and total metals.

**Correlation of Predicted Toxicity with Dissolved vs Total Metal in Water**

In general, dissolved and total metals in water show similar relationships to the expected water toxicity (based on effluent toxicity tests and reach dilution factors).

#### *5.2.3.2 H10 - Correlation of Biological Response with Periphyton Metal vs Dissolved Metal in Water*

Tables showing the correlation coefficients between water or periphyton chemistry and various biological measurements are provided in Appendix 3. Based on the magnitudes of the significant correlation coefficients, the key findings regarding hypothesis H10 are outlined below.

**Correlation of Community Structure with Periphyton Metals vs Dissolved Metals in Water**

The correlations are negative for fish CPUE and BPUE (slightly stronger for BPUE in general) and for number of fish taxa. In other words, CPUE, BPUE and fish taxa tend to decrease with increasing metal concentrations, either dissolved in water or (for CPUE and BPUE) associated with periphyton.

The correlations are negative for number of benthic taxa and EPT taxa, but positive for dominance of pollution-tolerant taxa such as *Rheocricotopus* and Orthocladiinae. In other

words, as metal concentrations increase in water or periphyton, the number of benthic and EPT taxa decreases and pollution-tolerant species comprise a greater percentage of total organism density.

The relationships are generally stronger (often substantially so) for metal in water than for periphyton metal, with a few exceptions such as brook trout CPUE and BPUE in relation to lead and aluminum.

While the hypothesis is not tested with total metals specifically, the results obtained for dissolved metals and total metals would be similar, as dissolved metal and total metal results are generally similar in terms of spatial trend and based on the results of H9.

**Correlation of Predicted Toxicity with Periphyton Metals vs Dissolved Metals in Water**

In general, dissolved metal in water shows a slightly stronger relationship to the expected water toxicity (based on effluent toxicity tests and reach dilution factors) than does the periphyton metal. This is true for Al, Cd, Cu and Zn whereas, for other metals (Pb, Fe), periphyton metal generally shows a slightly stronger relationship to expected water toxicity.

#### *5.2.3.3 H12 - Metal vs Metallothionein in Fish (Viscera) as a Biological Response to Environmental Metals*

Tables showing the correlation coefficients between metal or metallothionein in fish viscera, and metals in water (dissolved) or periphyton, are provided in Appendix 3. Based on the magnitudes of the significant correlation coefficients, the key findings regarding hypothesis H12 are outlined below.

For exposed wild Atlantic salmon, metallothionein in tissue is correlated with metals in water. The only significant correlations between corresponding metals in tissue and water are negative (and thus possibly spurious). These results indicate that the marginally insignificant exposure area trends in MT levels in wild salmon in H4 (Section 5.2.1) may be more

attributed to the use of the reach identifier rather than metal concentration in water in that analysis. The absence of correlations between metals in water and viscera is consistent with the absence of spatial trends in H4 for nearly all metals (except one).

For exposed blacknose dace, metallothionein is related only to lead in water, and zinc, copper and lead in tissue are related only to corresponding metals in periphyton. These results are consistent with the presence of an exposure area trend and reference-exposure difference in MT, and with the reference-exposure differences in metal levels in viscera and in periphyton.

For caged Atlantic salmon, metallothionein is correlated with metals in water and no correlations were seen between metals in water and metals in viscera. These correlations are consistent with responses seen in H4 (Section 5.2.1).

On balance, tissue metallothionein and tissue metal concentrations were both correlated with environmental metal concentrations in some instances, although neither metallothionein nor metals responded in a consistent manner among the fish tested.

### 5.2.4 H13 - Correlation of Biological Response with Predicted Effluent Toxicity

Figures illustrating the relationships between biological response and expected water toxicity (based on effluent toxicity and reach dilution factors), and tables showing the correlation coefficients, are provided in Appendix 3. Based on the magnitudes of the significant correlation coefficients, the key findings regarding hypothesis H13 are outlined below.

In general, the data show that the expected water toxicity (% inhibition) is negatively correlated with number of fish taxa, fish CPUE and BPUE, and Atlantic salmon CPUE and BPUE. The correlations involving brook trout and blacknose dace were not generally significant.

The data show that the expected water toxicity (% inhibition) is positively correlated with pollution-tolerant benthic taxa such as *Rheocricotopus* and Orthocladiinae. The correlations involving number of benthic taxa, number of EPT taxa and benthic density were negative but not significant.

### 5.2.5 Triad Hypotheses

There are many combinations of chemistry (C), toxicity (T) and biology (B) monitoring tools that show significant correlations on all three arms of the "triad". This is true whether we use dissolved or total metal chemistry in water, or periphyton chemistry. The strongest C-T, C-B and T-B correlations are listed in Appendix 3.

In the absence of sediment at this site, only water toxicity values are available, and these values are estimated for the exposure reaches based on effluent toxicity testing. Although triad analysis might be possible based on water toxicity tests on field-collected water samples, it is questionable whether it should be performed using estimated water toxicity values. Consequently, we have not performed any further statistical evaluations of the triad hypothesis at this site.

# 6.0 EVALUATION OF AQUATIC EFFECTS TECHNOLOGIES

## 6.1 Introduction

The Heath Steele program evaluated several of the aquatic effects monitoring "tools" considered by AETE. These tools were evaluated through testing eight of the thirteen hypotheses pertinent to the 1997 field program, as well as by examination of other tool performance indicators other than those specific to these hypotheses (e.g., other apparent cause-effect relationships, practical aspects, etc.). To avoid repetition, the cost-effectiveness aspects are considered collectively in the summary report on all four 1997 field sites, because costs for each specific technology were approximately equal at the four sites (BEAK and GOLDER, 1998b).

Monitoring tools may be organized within "tool boxes" under the four guiding questions formulated under the AETE program to develop the hypotheses tested (from Section 1.1):

1. Are contaminants getting into the system?
2. Are contaminants bioavailable?
3. Is there a measurable (biological) response? and
4. Are contaminants causing the response?

Tool boxes and monitoring tools may be categorized under these four questions. Some tools may logically fit under more than one question; for example, toxicity testing tools may fit under Questions 1, 2 or 3. Table 6.1 provides a reasonable framework for organization of these tools, although alternate frameworks may be equally valid.

The fourth question cannot be answered by the application of individual tools, unlike the first three questions. Rather, the fourth question can be answered only by integrating the use of tools between and among tool boxes through testing for statistical linkages between potential cause and effect variables (e.g., do chemical concentrations and biological measurements correlate with one another?). The most effective tools are clearly those used in combinations that provide a yes response to Question No. 4.

TABLE 6.1: GUIDING QUESTIONS, TOOL BOXES AND TOOLS CONSIDERED IN THE 1997 FIELD PROGRAM. TOOL BOXES AND TOOLS IN BOLD PRINT ARE SPECIFICALLY CONSIDERED AT HEATH STEELE.

| Question | Tool Boxes | Tools |
|---|---|---|
| Are contaminants getting into the system? | **Water chemistry** | • **total metal concentrations**<br>• **dissolved metal concentrations** |
| | **Sediment chemistry**[1] | • **total metal concentrations**<br>• partial metal concentrations |
| Are contaminants bioavailable? | **Fish tissues** | • **organ/tissue metal concentration**<br>• **organ/tissue metallothionein concentration** |
| Is there a measurable response? | **Effluent chronic toxicity**[2] | • **fathead minnow survival and growth test**<br>• ***Ceriodaphnia dubia*** **(microcrustacean) survival and reproduction test**<br>• ***Selenastrum capricornutum*** **(algae) growth test**<br>• ***Lemna minor*** **(duckweed) growth test** |
| | Sediment toxicity | • *Chironomus riparius* (larval insect) survival and growth test<br>• *Hyalella azteca* (crustacean) survival test<br>• *Tubifex tubifex* (aquatic worm) survival and reproduction test |
| | **Fish health indicators** | • **fish growth (length, weight and age)**<br>• fish organ size |
| | **Fish population/community health indicators** | • **fish catch-per-unit-effort (CPUE - by species and total)**<br>• **fish biomass-per-unit-effort (BPUE - by species and total)** |
| | **Benthic community health indicators** | • **densities of benthic invertebrates**<br>• **numbers of benthic invertebrates**<br>• **benthic community indices (e.g., EPT index)**<br>• **frequency of chironomid deformity** |
| | **Periphyton community health indicators** | • **periphyton community biomass**<br>• **numbers of periphyton taxa** |
| Are contaminants causing the response? | **Pair-wise combinations of the above tool boxes** | • **chemistry x biology tool correlations**<br>• **toxicity x biology tool correlations**<br>• chemistry x toxicity tool correlations |

[1] Periphyton metal concentration used as a surrogate for sediment metal concentration as a predictor of benthic effects at Heath Steele.

[2] Effluent chronic toxicity measured in the laboratory may also be categorized under Questions 1 or 2 (Are contaminants getting into the system?, or, Are contaminants bioavailable?).

The hypotheses are formulated to answer two general types of questions:

- Is the tool effective in measuring a mine effect (i.e., is there a reference - exposure difference or an exposure area gradient)?; and
- Is one tool more effective than another in measuring an effect?

The "effectiveness" of monitoring tools as discussed herein is specific to the Heath Steele data set. Heath Steele represents one of four mine sites considered in the AETE 1997 Field Program, and only one of dozens of mine sites across Canada. A tool that is found to be of little value at Heath Steele for detecting mine effects may be very useful at other sites and vice versa. Therefore, the reader is cautioned not to assume that the conclusions drawn with Heath Steele data will necessarily be broadly valid at mines across Canada. As shown in the AETE 1997 Field Program Summary Report, monitoring tools can respond very differently from site to site (BEAK and GOLDER, 1998b). Also, the presence or absence of a particular mine-related effect may simply reflect exposure level or bioavailability at the site. In the latter case, the absence of an effect may simply indicate that the tool was suitable for showing no effect. However, the degree of impact known to occur at Heath Steele and the aqueous concentrations of metals typically present are consistent with conditions which should demonstrate the effectiveness of monitoring tools unless they are insensitive.

## 6.2 Are Contaminants Getting Into the System?

### 6.2.1 Water Chemistry Tool Box

***Hypothesis Testing Aspects***

At Heath Steele, water chemistry sampling showed that metals were "getting into the system" in the vicinity of the mine's monitoring station, HS-3. This was demonstrated by a downstream gradient in total and dissolved concentrations of zinc, copper, lead, cadmium and aluminum. A gradient was also observed for lead and possibly iron concentrations in periphyton, but gradients were generally not evident for other metals in periphyton.

In testing of Hypotheses H9, H10 and H12, measured aqueous concentrations of metals from the Heath Steele site were effectively correlated with fish community health indicators (CPUE, BPUE, number of fish taxa), benthic community health indicators (density, numbers of taxa, EPT index) and some visceral metal and MT concentrations in wild Atlantic salmon and blacknose dace.

However, metal concentrations in water were more strongly correlated with visceral MT than with visceral metal concentration in all fish examined.

Biological (fish and benthic) community responses were similarly correlated with dissolved metal concentration and total metal concentration in water in H9. Total metals in periphyton were generally more weakly correlated with biological responses than were metal concentrations in water in H10.

***Other Considerations***

The collection of dissolved metal water samples according to the methods described in Annex 1 was not onerous, but required approximately five technician hours (additional relative to total metal samples) to filter and preserve the 18 samples (16 plus 2 duplicates) and appropriate filter blanks.

The syringes used, based on recommendations by chemists at the Geological Survey of Canada (GSC), were difficult to procure in Canada. Importation of the syringes from the U.S. required over one month due to delays at Canada Customs; thus, syringes were borrowed from GSC until delivery of the order.

The commercial laboratory used required very specific instruction to provide sampling containers and filtration materials consistent with the specifications provided by GSC. For example, commercial laboratories often provide low density rather than high density polyethylene containers for metal samples, and may also provide containers with coloured lids such as "Falcon" tubes to consultants or mining companies. GSC has shown that such containers can contribute low levels of metals to water samples, and thus may not be suitable in aquatic effects monitoring where metal concentrations of interest are equal to or often below surface water quality guidelines.

The filtration procedure involved squeezing the water through a syringe-mounted filter, and was somewhat difficult and time-consuming due to the slow rate of filtration, rinsing requirements, etc. Also, where suspended solids levels are higher (generally not at Heath Steele), filters became quickly clogged and required replacement.

Although no significant sample contamination was apparent in the dissolved metal results and in the filter blanks (i.e., dissolved metal concentrations were generally less than or equal to total metal concentrations and blanks were generally below detection limits), a greater potential for

sample contamination exists in the field for dissolved metals than for total metals owing to the handling required. Some contamination was apparent at one of the other three sites.

To conclude, water chemistry (metal concentration) measurements were effectively correlated with biological effects in fish and benthos at Heath Steele. Dissolved and total metal concentrations were similarly correlated with biological effects, and water concentrations were more often and more strongly correlated with biological effects than were periphyton metal concentrations.

### 6.2.2 Sediment (Periphyton) Chemistry Tool Box

***Hypothesis Testing Aspects***

The periphyton metal tool, as a possible surrogate for sediment metals, has been evaluated by identifying reference versus exposure differences or concentration trends within the exposure gradient, and by examination of periphyton as a possible causal agent for biological responses (H10, H12).

In general, reference-exposure differences in periphyton metal concentrations were observed for zinc, lead, cadmium and copper, and exposure area trends for lead and iron. These trends could, in part, be due to the effects of metal precipitates (e.g., iron hydroxide) collecting on the stream substrate (and forming part of the periphyton samples).

Periphyton metal concentrations provided some linkages to biological effects. In particular, periphyton metal levels were correlated with benthic and fish community responses, as well as with some metal levels in blacknose dace viscera from the exposure area. Within exposure reaches, periphyton metal concentrations were generally better correlated with visceral metal concentrations than were aqueous metal concentrations in blacknose dace. This may, in part, be due to the fact that blacknose dace are known to feed on algal species (Scott and Crossman, 1973).

***Other Considerations***

At Heath Steele, variability in the periphyton growth forms and species present affected the biological composition of the sample collected and in turn probably influenced metal concentrations. Species composition and biomass varied substantially from site to site, potentially affecting metal bioaccumulation within any particular sample. Also, material other than algae may be present in the samples such as mineral precipitates and bacterial slimes. These factors may be more important in influencing metal concentrations in periphyton than in sediment material.

Periphyton was relatively easy to collect in the field. However, at some stations, growth was sparse and collection times of up to 10 to 15 minutes per sample were necessary. Although this collection time is not excessive, it generally exceeds the time needed to collect a water or sediment sample. Periphyton samples, however, required minimal preparation after collection (label and freeze only).

Periphyton metal concentrations may be most useful in identifying whether contaminants are "getting into the system" in special cases where aqueous metal concentrations are affected only sporadically (e.g., only in response to runoff or to intermittent effluent discharge), with concentrations approaching natural background between these impact events. In cases where soft sediments are sparse or absent (e.g., a fast-flowing river), periphyton metal concentrations may be effective in integrating the ranges of water quality conditions prevailing in the recent past.

## 6.3 Are Contaminants Bioavailable?

### 6.3.1 Tissue Metal Concentrations

***Hypothesis Testing Aspects***

At Heath Steele, the effectiveness of visceral metal concentration as an indicator of metal bioavailability is measured from the identification of differences in concentrations between reference and exposure areas and/or the occurrence of linear trends within the exposure reaches. Effectiveness is also determined by the strength of correlations between possible causal agents (metals in water or periphyton) and metals in viscera.

Reference area-exposure area differences in visceral metal concentrations were observed for lead in wild Atlantic salmon and copper and zinc in blacknose dace, although no concentration trends were seen in either species in the exposure zone. Reference-exposure area differences were also

apparent for visceral copper and cadmium in brook trout, with a possible exposure area trend indicated for copper.

Visceral metal concentrations in wild fish were not correlated with exposure to metals in water. However, blacknose dace Cu, Pb and Zn in viscera were correlated with concentrations of the same metals in periphyton, reflecting a possible food chain linkage.

Metal bioavailability was not indicated by visceral metal concentrations in caged Atlantic salmon exposed for nine days downstream of Heath Steele. The reduction or elimination of feeding by these fish in the cages may have reduced any food intake pathway for metals, and the exposure time may have been inadequate to induce the effect seen in wild fish. However, because MT results suggest that metal exposure has occurred, the absence of apparent response in metal concentration might be attributed to homeostatic processes in the viscera, potentially involving MT.

Gill in caged salmon appeared to respond to metal exposure, with gill cadmium levels in particular paralleling the aqueous cadmium gradient present. Although Hypothesis 2 was not specifically intended for testing at Heath Steele, the results of gill and visceral analyses in caged fish imply that at least for short-term metal exposures, analysis of metals in gill may be a better indicator of metal bioavailability than metals in viscera.

***Other Considerations***

From a practical standpoint, processing of small fish for metal analysis is accomplished more quickly than required for larger fish at other mine sites. Small fish samples were easily processed in the field simply by freezing the whole fish on dry ice. These fish were dissected and the tissues processed and analyzed for metals and MT by the Department of Fisheries and Oceans, Winnipeg, Manitoba. At most Heath Steele sites, small fish were abundant and could be sampled quickly by electrofisher during one visit to each station (versus use of gillnets for larger fish).

It is not certain to what extent visceral analysis is influenced by the quantity of food present in the gut of the fish. However, one might assume that visceral metal concentration may be either reduced or increased in viscera following feeding periods, depending on metal concentrations in the food consumed. For example, the correlation between periphyton metal concentrations and metal concentrations in blacknose dace viscera may reflect either bioaccumulation by the tissues or

simply the presence of periphyton in the alimentary canal. This possible source of variation would not occur in the analysis of individual organs or tissues.

### 6.3.2 Tissue Metallothionein Concentrations

***Hypothesis Testing Aspects***

The effectiveness of visceral MT concentration as an indicator of metal bioavailability is measured as described above for visceral metals (i.e., by reference-exposure area differences, exposure area trends and correlations with possible causal agents). A reference-exposure difference and/or an exposure area trend indicate an effective tool response.

Reference area-exposure area differences in visceral MT were evident in blacknose dace viscera, with a linear trend indicated for caged salmon. The reference area-exposure area difference was weak and not significant for wild salmon ($p = 0.099$). The exposure area trend for blacknose dace was also weak and not significant ($p = 0.06$).

Visceral MT levels responded to exposure concentrations in water for exposure area wild salmon for most metals of concern. Visceral MT levels in caged salmon were strongly correlated with aqueous metal concentrations. Correlations between exposure concentrations of metals and visceral MT were found in blacknose dace only for lead.

Although Hypothesis 3 was not specifically intended for testing at Heath Steele, the analysis of both gill and viscera in caged Atlantic salmon appears to indicate that, for short-term exposures, both tissue types responded similarly to exposure. However, gills from two fish were necessary to provide sufficient mass for a single sample for analysis of MT and metals (in contrast to viscera from a single fish).

***Other Considerations***

The collection of fish for MT analysis at Heath Steele was accomplished readily and fish required little processing, as described in Subsection 6.3.1. Maintenance of a reliable supply of dry ice required for preservation of tissues for MT analysis was somewhat problematic at Heath Steele owing to the absence of a commercial supply in Miramichi and a requirement for courier deliveries from Halifax, Nova Scotia. Maintenance of a dry ice supply required daily diligence to confirm orders and to track deliveries. Also, a dry ice quantity limitation of 2 kg on passenger aircraft required an unscheduled re-packaging of samples at the airport and a requirement for immediate replenishment of the dry ice supply upon sample arrival at BEAK.

### 6.3.3 Tissue Metal vs Metallothionein Comparison

Based on the Heath Steele data, MT and metal concentrations in wild fish viscera and caged fish gill both responded to metal exposure. Visceral MT was better correlated with metal concentrations in the environment than were visceral metals in salmon, while the reverse was true for blacknose dace. For shorter-term exposure using salmon, visceral MT was more effective than visceral metal concentration.

The correlation matrix of tissue metal versus metallothionein concentrations in fish viscera and gill suggests that some metals are more closely associated with tissue metallothionein than others. For fish at Heath Steele, the highest correlation coefficients occur for cadmium in most cases where metal-metallothionein relationships were apparent. This suggests that MT is likely more effective in reflecting the bioavailability of some metals than others.

## 6.4 Is There A Measurable Effect?

### 6.4.1 Effluent Chronic Toxicity

***Hypothesis Testing Aspects***

Estimates of *in situ* chronic toxicity, based on effluent toxicity test results and a Tomogonops River dilution model, were correlated with *in situ* effects in some fish community and benthic community indices. The three most sensitive tests (*Ceriodaphnia, Selenastrum* and duckweed) were more effective than the least sensitive (fathead minnow) test. This ability to predict

biological effects with Heath Steele toxicity results is intuitively reasonable, because chronic effect endpoints occurred at effluent concentrations also found along the exposure gradient.

Predicted *in situ* chronic toxicity was also strongly correlated with measured metal concentrations in water. Thus, a strong cause-effect linkage is implied between water concentrations and chronic toxicity, as well as between chronic toxicity and in-stream response.

***Other Considerations***

Of the four tests, *Selenastrum* and *Ceriodaphnia* were the most sensitive to Heath Steele "effluent", and fathead minnow was the least sensitive. All tests effectively measured chronic toxicity except in one case (June 1997 sample) when no lethal or sublethal effect was measured in fathead minnow.

As documented in the Summary Report (BEAK and GOLDER, 1998b), similar toxic responses were obtained in chronic testing of *Ceriodaphnia* and fathead minnow using Heath Steele site dilution water and laboratory dilution water having a hardness similar to site water. Thus, for Heath Steele, little or no reduction in toxicity was achieved in site dilution water, in spite of the presence of potential modifying factors such as dissolved organic carbon (typically 3 to 5 mg/L) in site water.

Testing of H13 as worded could have been undertaken more directly by measuring chronic toxicity in water collected from each downstream exposure station. In this way, linkages between causal agents (toxicity) and biological response would be based on data from the site rather than from toxic responses predicted indirectly from testing of effluent. In practice, however, this alternate approach would have been problematic owing to difficulties in discerning responses at effluent concentrations that in many cases would have been low relative to effect concentrations, and to the dilution water effect (as noted below) which would have been inherent in the samples.

Use of site dilution water was associated with invalid test results for fathead minnow in two of the three Heath Steele tests, as well as in some of the fathead minnow tests completed for the other mines in this program. This condition arose from excessive mortalities in control fish, apparently in response to fungal growth on the fish.

In terms of the practical aspects of the testing, use of site dilution water added a level of difficulty to test logistics. In particular, use of site dilution water added to the acclimation requirements for

fathead minnow and *Ceriodaphnia*, and necessitated additional sampling effort and shipping expense.

### 6.4.2 Fish Growth

***Hypothesis Testing Aspects***

In neither juvenile Atlantic salmon nor in blacknose dace was fish size at age related to effluent exposure. The only reference-exposure difference seen in size at age was in the occurrence of larger Atlantic salmon in the exposure area than in the reference area, possibly owing to greater density effects (competition) in the reference area. This result is interesting in light of the evidence for other effects on fish at the population and community levels in that fish apparently are diminished in numbers in exposure areas, but the fish present in exposure areas are not impaired in terms of growth.

### 6.4.3 Fish CPUE and BPUE

***Hypothesis Testing Aspects***

CPUE or BPUE for all fish species combined effectively responded to effluent exposure, as measured in H5, H6 and H9. The CPUE and BPUE tools were similarly effective for juvenile Atlantic salmon among comparable stations, but not for other individual species such as blacknose dace or brook trout. CPUE and BPUE were particularly effective in showing a spatial trend consistent with the aqueous metal gradient in the exposure area.

***Other Considerations***

CPUE and BPUE were readily measured in the field, with two monitoring stations completed by a crew of two each day. This effort included often difficult site access, as well as the identification and processing of all fish captured.

### 6.4.4 Benthic Community Health Indicators

***Hypothesis Testing Aspects***

Monitoring of benthic community parameters was effective in identifying metal exposure responses in the exposure area at Heath Steele, with effects on numbers of taxa, EPT index and numbers of specific species evident. This effectiveness was evident in terms of reference-exposure differences and with respect to correlations with aqueous metal concentrations. Total benthic density was not effective in distinguishing an exposure effect, because metal-tolerant forms replaced more sensitive species in exposure areas.

Benthic indices could be predicted based on metal concentrations in the water. This strengthens our conclusion that the response is associated with metal exposure. Weaker associations were seen between benthic indices and periphyton metals, suggesting that effects are predominantly associated with exposure to aqueous metals.

***Other Considerations***

The incidence of chironomid abnormality, based on examination of mouth parts in mounted specimens, was low throughout the reference and exposure area (Appendix 5), indicating that this tool would be ineffective in measuring biological responses to metals at Heath Steele.

The presence of a very rich and diverse benthic fauna in the Tomogonops River probably enhances the sensitivity of benthic monitoring to metal exposure. Similar trends can be more difficult to discern in aquatic habitats supporting a less diverse benthic assemblage.

### 6.4.5 Periphyton Community Indicators

***Hypothesis Testing Results***

Periphyton densities and numbers of taxa did not show a reference-exposure area difference or a trend consistent with a response in the exposure gradient. Based on this result, periphyton community conditions were ineffective in monitoring exposure at Heath Steele.

***Other Considerations***

Periphyton communities may be affected by degree of shading by riparian vegetation, substrate type, flow conditions and other aspects of habitat. Monitoring of periphyton on artificial substrates (colonization surfaces) could be more effective than natural community monitoring, because it would provide a standard surface for colonization. However, this would necessitate a return field trip to retrieve the substrates. Monitoring of natural benthic communities offers a clear advantage over periphyton as a biomonitoring tool in terms of cost effectiveness, ease of sample collection and environmental relevance.

## 6.5 Are Contaminants Causing the Responses?

As indicated previously, this question is not answered directly through the application of specific monitoring tools evaluated in this study, or through any of the hypotheses tested. Rather, the question is evaluated only by a weight-of-evidence provided by affirmative responses to the first three questions, and particularly by the strength of correlations between exposure indicators (chemical concentrations) and biological responses in hypotheses H9 through H13.

At Heath Steele, evidence indicates that contaminants are getting into the system and are bioavailable, and that certain biological responses are correlated with metal concentrations in the environment. Certain fish community and benthic community responses are correlated with aqueous concentrations of metals in the Tomogonops River, and the directions of exposure-response relationships are consistent with biological effects. Furthermore, *in situ* toxicity predicted from laboratory toxicity testing is also correlated with these biological effects. Accordingly, the field data support a conclusion that "contaminants are causing the responses". However, dose-response relationships in the field do not necessarily prove cause and effect. Rather, a combination of controlled laboratory testing of metal toxicity and field evidence such as provided herein would be appropriate to provide further detail on cause and effect (e.g., which metals individually or in combination produce a response).

## 6.6 Section Summary

Table 6.2 provides a summary of the effectiveness rankings of the aquatic monitoring tools evaluated at Heath Steele. Table 6.3 compares the effectiveness of alternate tools that may be used to measure metal concentrations, metal bioavailability or biological response.

For Table 6.2, the rankings are based on those statistical indicators of mine effect available for each tool (reference-exposure differences, exposure area trends, etc.). The "Effect Detected" ranking is used when effects are consistently measured for a tool at Heath Steele. An "Effect not Demonstrated" ranking means that effects are never measured with the tool in question at Heath Steele. The "Effect Partially Demonstrated" category applies when effects are measured in only some cases with a particular tool. For example, statistically significant MT responses in fish occurred in blacknose dace and caged juvenile salmon but not in wild salmon. Thus, MT in viscera demonstrated a partial effect.

Overall, most of the tools evaluated were effective or somewhat effective. Periphyton community structure, fish growth and benthic community density were ineffective. Of those tools that were effective, some were more effective than others as predictors of biological response.

TABLE 6.2: EFFECTIVENESS OF MONITORING TOOLS TESTED AT HEATH STEELE (Note: Refer to Table 6.3 for tool comparisons)

| Tool Boxes | Tools | Effectiveness | | | Comment |
|---|---|---|---|---|---|
| | | Effect Demonstrated | Effect Partially Demonstrated | Effect Not Demonstrated | |
| Water Chemistry | Total Metals | √ | | | Gradient in Zn, Cd, Cu, Pb and Al in exposure area. Correlated with biological effects and tissue MT and metal levels. |
| | Dissolved Metals | √ | | | Gradient in Zn, Cd, Cu, Pb and Al in exposure area. Correlated with biological effects and tissue MT and metal levels. |
| Sediment (Periphyton) Chemistry | Periphyton Metals | | √ | | Gradient in exposure area evident for Pb and Fe. Some correlations occurred between periphyton metals and biological/tissue responses. |
| Fish Tissues | Visceral Metals | | √ | | Visceral metal levels responded to metal exposure (increase in exposure area fish) but little or no trend present in exposure area. Visceral metals in caged salmon did not respond to metal gradient. |
| | Gill Metals | | √ | | Gill metals responded effectively to exposure over nine days in caged salmon, especially for Cd. Spatial trends not well developed for other metals. |
| | Visceral MT | | √ | | Visceral MT levels responded to metal exposure (increase in exposed fish) in blacknose dace. Visceral MT showed clear spatial trend in exposed caged salmon, but no significant response seen in wild salmon. |
| | Gill MT | √ | | | Gill MT showed clear spatial trend in exposed juvenile salmon (caged). |
| Effluent Toxicity | *Ceriodaphnia* | √ | | | *Ceriodaphnia* and *Selenastrum* were the most sensitive tests. All tests were correlated with some *in situ* benthic/fish effects. |
| | *Selenastrum* | √ | | | *Ceriodaphnia* and *Selenastrum* were the most sensitive tests. All tests were correlated with some *in situ* benthic/fish effects. |

TABLE 6.2: EFFECTIVENESS OF MONITORING TOOLS TESTED AT HEATH STEELE (Note: Refer to Table 6.3 for tool comparisons)

| Tool Boxes | Tools | Effectiveness | | | Comment |
|---|---|---|---|---|---|
| | | Effect Demonstrated | Effect Partially Demonstrated | Effect Not Demonstrated | |
| Effluent Toxicity (cont'd) | *Lemna minor* | √ | | | *Ceriodaphnia* and *Selenastrum* were the most sensitive tests. All tests were correlated with some *in situ* benthic/fish effects. |
| | Fathead minnow | | √ | | Fathead minnow was the least sensitive of the four tests and presented difficulties in acclimation to site water. |
| Fish Health Indicators | Growth | | | √ | No effects on growth seen in sentinel species. |
| Fish Population/ Community Health Indicators | CPUE/BPUE | √ | | | Catch and biomass of fish per unit effort effective in responding to exposure. Total community results (all fish) affected more clearly than individual species. |
| Benthic Community Health Indicators | Benthic Density | | | √ | Exposure-reference difference or exposure trend not evident. |
| | No. of Taxa | | √ | | Exposure-reference difference evident; exposure area trend apparent but not significant. |
| | No. of EPT Taxa | √ | | | • Exposure-reference difference evident<br>• Exposure area trend evident and significant. |
| | Abundances of Indicator Species | √ | | | • Exposure-reference difference evident.<br>• Exposure area trend evident and significant. |
| Periphyton Community Health Indicators | Periphyton Biomass | | | √ | Reference-exposure area differences or exposure area trends not observed. |
| | No. of Periphyton Taxa | | | √ | |

TABLE 6.3: COMPARATIVE EFFECTIVENESS OF MONITORING TOOLS AT HEATH STEELE

| Tools | Comparison |
|---|---|
| Total Metals vs Dissolved Metals in Water | Dissolved metal and total metal concentrations were similar in terms of strength of correlation with biological responses. |
| Metals in Water vs Metals in Periphyton | Metals in water rather than periphyton were more strongly correlated with community level biological responses on balance. Periphyton Cu, Zn and Pb were better correlated with visceral metals in blacknose dace than were aqueous metals, possibly reflecting periphyton in the gut. |
| Visceral Metals vs Visceral MT in Fish | On balance, MT responded more frequently or strongly to exposure than did metals in small fish viscera. Effectiveness differed greatly from species to species and MT only responded in caged salmon. The responsive metals variously included Zn, Pb, Cu and Cd. |
| Gill Metals vs Gill MT in (Caged) Salmon | Gill metals were variable in responsiveness to exposure (Cd most effective); gill MT was effective. |
| Visceral Metals vs Gill Metals in (Caged) Salmon | Gill concentrations of some metals were responsive to exposure. Visceral metals were not. |
| Visceral MT vs Gill MT in (Caged) Salmon | Visceral and gill MT appeared equally responsive to exposure. |
| Effluent Chronic Toxicity Tests | All tests were effective in predicting in-stream effects on natural communities; fathead minnow test was the least sensitive of the four. |
| Fish CPUE/BPUE (individual species vs whole community) | CPUE and BPUE were responsive to exposure; CPUE and BPUE were more responsive at the community level (all fish) than at the individual species level. |
| Benthic Community Health Indicators (density, no. of taxa, EPT index, indicator taxa) | Benthic EPT index and abundances of some indicator species were more responsive than numbers of taxa to exposure. Total densities did not respond effectively. Only a selected subset of indices was tested (beyond core hypotheses requested by AETE). |

# 7.0 REFERENCES

Assessment of the Aquatic Effects of Mining in Canada (AQUAMIN). 1996. Final Report. Prepared for AQUAMIN Steering Group. Prepared by AQUAMIN Working Groups 7 and 8.

Beak Consultants Limited (BEAK). 1996. 1995 Field Evaluation of Aquatic Effects Monitoring Methods. Report prepared for Natural Resources Canada, CANMET. Ottawa, Ontario.

Beak International Incorporated (BEAK). 1997. Heath Steele Mine Closure Plan. Part 1: Tailings Basin. Support Document 2: Current Environmental Conditions. Report prepared for Noranda Mining and Exploration Inc., Heath Steele Division.

Beak International Incorporated (BEAK). 1998a. 1997 Field Program - AETE. Dome Mines Site Report. Report prepared for Natural Resources Canada, CANMET. Ottawa, Ontario.

Beak International Incorporated and Golder Associates Ltd. (BEAK and Golder). 1998b. Summary and Cost-Effectiveness Evaluation of Aquatic Effects Monitoring Technologies Applied in the 1997 AETE Field Evaluation Program. Report prepared for Natural Resources Canada, CANMET. Ottawa, Ontario.

Beak International Incorporated and Golder Associates Ltd. (BEAK and Golder). 1997. Study Design and Plan for 1997 Field Studies. Report prepared for Natural Resources Canada, CANMET. Ottawa, Ontario.

Borenstein, M. and J. Cohen. 1988. Statistical Power Analysis: A Computer Program. Lawence Erlbaum Associates, Inc.

Canadian Council of Resource and Environment Ministers (CCREM). 1987. Canadian Water Quality Guidelines.

Environment Canada. 1992a. Biological Test Method: Test of Reproduction and Survival Using the Cladoceran *Ceriodaphnia dubia*. Environment Canada EPS 1/RM/21.

Environment Canada. 1992b. Biological Test Method: Test of Larval Growth and Survival Using Fathead Minnows. Environment Canada EPS 1/RM/22.

Environment Canada. 1992c. Biological Test Method: Growth Inhibition Test Using the Freshwater Alga *Selenastrum capricornutum*. Environment Canada EPS 1/RM/25.

EVS Environment Consultants, Ecological Services for Planning and Jacques Whitford Environment Limited. 1997. Field Evaluation of Aquatic Effects Monitoring. 1997 Study Design. Final Report. Report prepared for Natural Resources Canada, CANMET. Ottawa, Ontario.

Finney, D.J. 1971. Probit Analysis. Cambridge University Press. 333 pp.

Rott, E. 1995. Diatoms of the Grand River, Ontario, Canada. Limnologica, 25: 165-192.

Saskatchewan Research Council (SRC). 1995. Annual Report. Development of Aquatic Plant Bioassays for Rapid Screening and Interpretive Risk Assessments of Metal Mining Wastewaters. Report prepared for Environment Canada.

Saskatchewan Research Council (SRC). 1996. Draft Protocol for the *Lemna minor* Growth Inhibition Test. A Modification of the 8211 Duckweed (Proposed) Toxicity Test Procedure published by American Public Health Association (1995).

Scott, W.B. and E.J. Crossman. 1973. Freshwater Fishes of Canada. Fisheries Research Board of Canada, Ottawa, Bulletin 184.